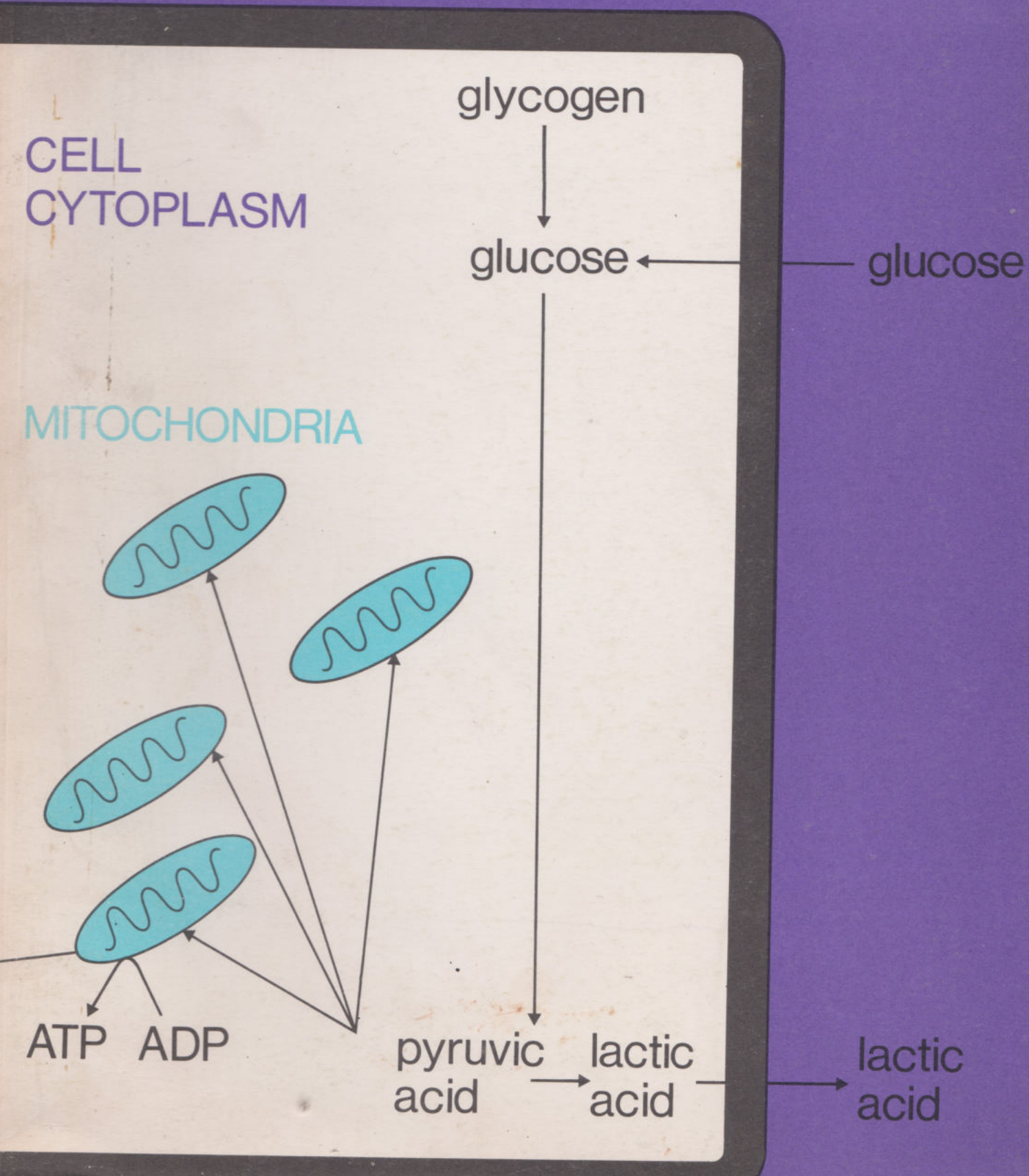
S100 15 and 16

THE OPEN UNIVERSITY

Science Foundation Course Units 15 and 16

Cell dynamics and the control of cellular activity



The Open University

Science Foundation Course Units 15 and 16

CELL DYNAMICS AND THE CONTROL OF CELLULAR ACTIVITY

Prepared by the Science Foundation Course Team

THE OPEN UNIVERSITY PRESS

A NOTE ABOUT AUTHORSHIP OF THIS TEXT

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The Open University Press
Walton Hall, Bletchley, Bucks

First Published 1971

Designed by The Media Development Group of the Open University

Printed in Great Britain by
Oxley Press Ltd,
London and Edinburgh

SBN 335 02007 0

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**Note: These two Units represent two weeks' work, but they are bound as one, and the division between them is arbitrary and not symmetrical: Unit 15 is longer than Unit 16.*

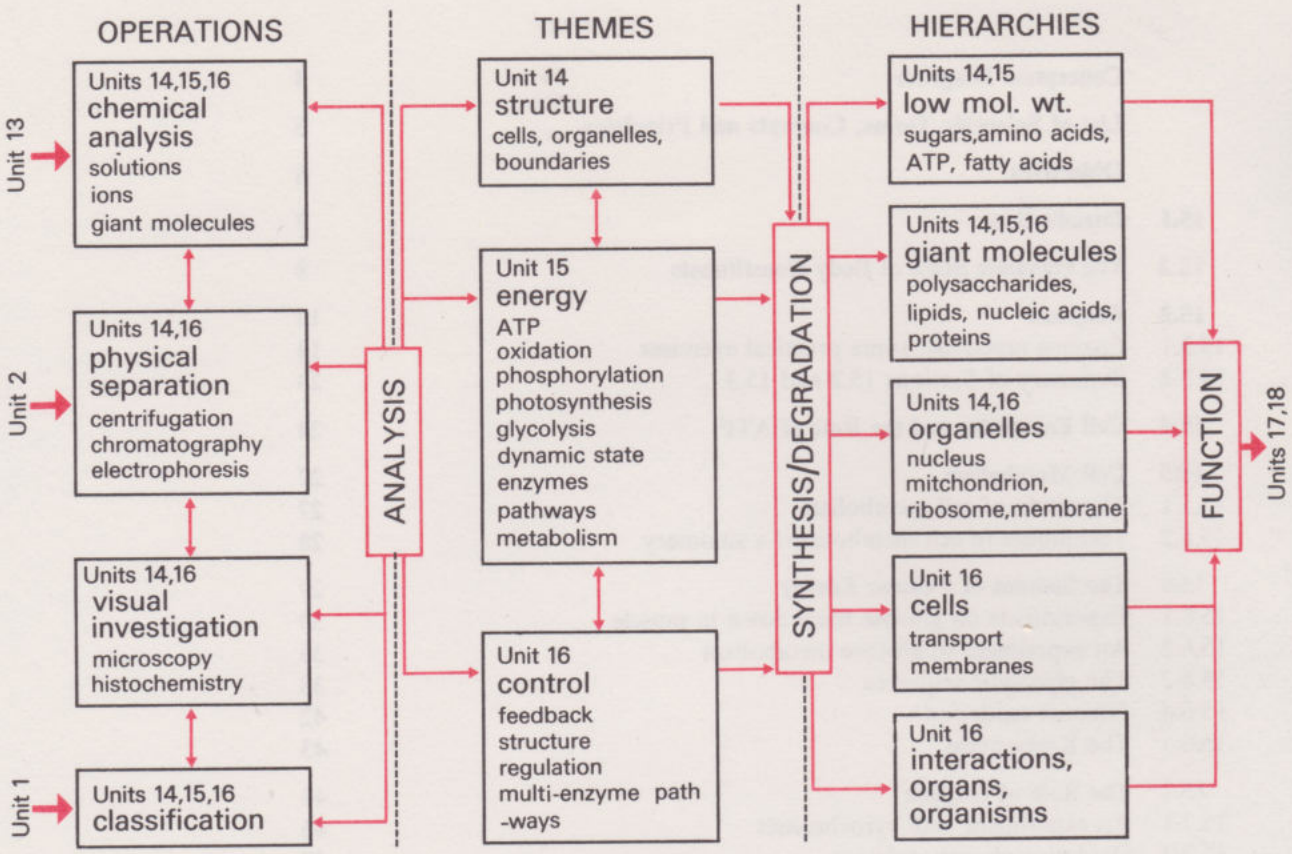
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Table A

A List of Scientific Terms, Concepts and Principles used in Units 15 and 16

Taken as pre-requisite			Introduced in this Unit			
1 Assumed from general knowledge	2 Defined in a previous Unit	Unit No.	3 Developed in these Units or in their set book	Page No.	4 Developed in a later Unit	Unit No.
vitamin	protein	13	steady state	8		
food	lipid	13	precursor	9		
digestion	nucleic acid	14	coenzyme	13		
poison	amino acid	10	enzyme	13		
yeast	fatty acid	14	specificity	13		
muscle	nucleotide	6	activator	14		
temperature	pH	9	active centre	14		
fermentation	relative molecular weight	6	inhibitor	14		
respiration	polypeptide	10	salivary amylase	14		
circulation of blood	(l) and (d) isomers	10	substrate	14		
virus	glucose	10	time course of reaction	14		
cartilage	carbohydrate	10	ADP	25		
saliva	isotope	6	ATP	25		
	radioactive	6	energy-poor	25		
	half-life	6	energy-rich	25		
	exponential	6	phosphorylation	26		
	catalysis	7	anabolic	27		
	colorimeter	7	catabolic	27		
	acid	9	metabolic pathway	27		
	buffer	9	metabolism	27		
	reaction velocity	11	aerobic	30		
	reversible reaction	11	anaerobic	30		
	activation energy	11	glycolysis	30		
	denaturation	14	glycolytic pathway	38		
	endothermic	11	Krebs cycle	43		
	exothermic	11	cell respiration	44		
	oxidation	8	dehydrogenase	45		
	reduction	8	electron transport	45		
	polysaccharide	10	glucose oxidation	45		
	hydrolysis	9	cytochrome	46		
	glycogen	14	oxidative phosphorylation	49		
	cell	14	photosynthesis	53		
	mitochondria	14	control processes	59		
	cytochrome	14	information theory	59		
	cytoplasm	14	active transport	60		
	ribosome	14	feed-back	60		
	cell membrane	14	lysosome	60		
	chloroplast	14	hierarchy	65		
	chlorophyll	14	holism	65		
	cellulose	14	reductionism	65		
	potential energy	4	vitalism	65		
	subcellular fractionation	14	end-product inhibition	TV		
			rate-limiting reactions	TV		

Any scientific terms used in this Unit but not listed are marked thus † and defined in the glossary.

Objectives

When you have completed the work for this Unit you should be able to:

1 Define, or recognize adequate definitions of, or distinguish between true and false statements concerning each of the terms, concepts and principles in column 3 of Table A.

2 Demonstrate your comprehension of material in the Units by being able to:

(i) recognize which of a given series of concepts concerning the major theories of *energy traffic*, *information traffic* and *structure* are implicitly involved in the context of given statements and to give reasons for the selection;

(ii) distinguish between warranted, unwarranted and contradicted conclusions drawn from a body of given data concerning the mechanism of enzyme action, the dynamic state of body constituents, the synthesis of ATP during glucose breakdown, the photosynthetic production of glucose and the factors limiting reaction rates in multi-enzyme systems.

3 Demonstrate your capacity for application of your knowledge by:

(i) applying procedures and principles drawn from earlier Units, especially Units 1 and 2, concerning induction, deduction and hypothesis-making, to material presented on the key experiments involved in the development of themes concerning: the dynamic state of body constituents, the history of cell respiration, glycolysis, biochemistry of muscle contraction, photosynthesis and the relation between enzymes and organelles;

(ii) utilize material drawn from the radio programmes associated with these Units to illustrate several ways in which recent biochemical advances, including development of pharmaceutical and chemical warfare agents, are affecting contemporary society.

4 Demonstrate understanding of the concept of hierarchy by being able, when presented with a list of problems in cell biology, to indicate at which level (atomic, molecular, intracellular, cellular) of the hierarchy they should best be approached experimentally.

5 Demonstrate the capacity to design, carry out, draw conclusions from and write up in acceptable form experiments concerned with (a) enzyme kinetics; (b) yeast metabolism.

15.1 Introduction

Units 15 and 16 explore the dynamic state of cellular constituents and the self-regulating nature of the cell system. Unit 15 takes as its theme *energy*. It shows first that the cell consists not of a static collection of molecules but of substances in dynamic interaction. Cell reactions depend on the existence of chemical catalysts—the enzymes. The properties of the enzymes are briefly described. Some of these cell reactions are *energy-requiring*, others are *energy-yielding*. This leads to a consideration of work and energy at the biological level and the central role of ATP as the biological energy currency. Some energy-yielding reactions, including glucose catabolism and photosynthesis, are described and the role of biological oxidations is discussed.

The second Unit, 16, takes as its major theme *control and regulation* of metabolism. To do this, we begin with examples of *synthetic* reactions, such as glycogen synthesis. The problem of the organization and control of these reactions can be explained in terms of cell structure and partition of enzymic function. Feedback mechanisms for regulation are discussed as examples of general systems.

A second theme for Unit 16 is how the cell acts upon its external environment; reference is made to muscle, nerve and secretory cells of the type whose function is discussed in Unit 18.

The experimental approach towards problems is emphasized in the text by means of structured exercises, designed to demonstrate the relationship between experimental design and conclusions, and in the television and radio programmes of both Units; one radio programme is devoted to the perils and promises of contemporary biochemistry.

Note: To help you focus attention on the major themes of the Units, summaries or study comments on each major section of the text are included.

15.2 The Dynamic State of Body Constituents

Study Comment

Within all the cells of the body there is a continuous and rapid process of breakdown and resynthesis of the molecules of which the cells are composed. This phenomenon is known as the 'dynamic state of body constituents'.

In this section, an investigation into this dynamic state is described and analysed by means of a structured exercise. Do not attempt to learn the details of the laboratory work described, but you should understand how the conclusions are derived from the results given.

The picture of the cell that we built up last week was one of precise and definite structures, made up of a multitude of complex chemicals. These structures and chemicals would seem likely to have equally precise functions. Their properties must relate in some way to the properties and activities of the cell as a whole. Yet the picture we have described until now is a static one; it is one of things rather than events, of photographs rather than ciné-films. In order to obtain this picture, it was necessary to destroy the living tissue at some arbitrary time, and subject it to drastic procedures – either to isolate individual chemicals, or to examine it microscopically, or to separate, by centrifugation, particular subcellular organelles.

We began Unit 14 by asking what characteristics distinguished a living from a dead organism. Yet, in attempting to answer this question by the analytical techniques of that Unit, we seem to have ignored just those things which make life different from death. Proteins, lipids and nucleic acids can be synthesized from amino acids, fatty acids and nucleotides in the test-tube; those molecules present in the dead organism are to all intents and purposes identical with those in the living ones. But no one would mistake a dead for a living organism. Where then, if not in the chemicals and the structure, does the difference lie?

In answering this question, we enter the second of the phases of research into the cell that we described in Unit 14, in which we are concerned not with the static picture of the cell but with the kinetics and dynamics of its existence. The highly organized molecules and structures, discussed in Unit 14, readily break down as soon as their environment alters beyond certain well-defined limits of pH and temperature. If life is to be preserved, any change that tends to alter such variables beyond these limits must be opposed. It follows that the cell (and the organism) has to maintain an internal constancy of structure and pH, and a chemical constancy so that breakdown of any substance is matched by the synthesis of a similar quantity of the same substance. Such a condition is known as a *steady state*. If this steady state were to break down at any point, and the conditions within the cell were to change by more than a small amount, the result could be both disastrous and irreversible. For instance, a change in pH sufficient to denature the cell proteins would irreversibly wreck the cell; it would, in fact, kill it.

steady state

A further characteristic distinguishes the chemical substances present within the cell. They are all extremely *unlikely* substances. A protein of relative molecular weight 34 000 has 288 amino acids in its polypeptide chain. If only 12 out of the 20 different naturally occurring amino acids are present, the number of possible isomers is 10^{300}. If only one molecule

of each isomer were to exist, the total mass would be some 10^{280} g. Compare this with the mass of the Earth, which is only 10^{27} g! Yet if the cell is to function, precisely the right protein must be present, not one 'nearly like it'. In the living cell, such molecules cannot arise purely by random chemical reactions; they must be built up according to specific pathways of synthesis with a precision far beyond that which can be achieved in the test-tube. There must be mechanisms within the cell which can distinguish between even such close relatives as the (l) and (d) isomers of amino acids (if you do not recall this terminology, check back with Unit 14), or between sugars such as glucose and galactose.

This problem of synthesis would not be so substantial if the synthesis were, so to speak, a once-for-all job, if the lipid, protein, and carbohydrate molecules only had to be made in the desired quantity, laid down in the appropriate structure, and then were able to go on functioning indefinitely until 'fair-wear-and-tear' demanded their replacement. But is this in fact the case? Consider the following experiment.* It was made in 1966 by the biochemist Abel Lajtha in New York. He was interested in estimating the time taken for the proteins of brain tissue to be broken down and re-synthesized again. He took a group of adult mice and added a known amount of the amino acid lysine to their food (the lysine is utilized in the animal to synthesize protein; it is a *precursor* of the protein). Lysine is present in all proteins, and the lysine in the animals' diet would therefore be expected to be incorporated into many different proteins as they were synthesized. The lysine used in this experiment was abnormal in one respect: its carbon atoms were not those of the normal ^{12}C isotope but of its radioactive counterpart ^{14}C (which has a half-life of 5 770 years—see Unit 6). Extraction of the tissue proteins and measurement of their radioactivity would therefore be a good measure of the amount of lysine which had been incorporated into protein, and hence of the total amount of lysine-containing proteins made.

turnover of proteins

Lajtha fed the mice with radioactive lysine for a week, mated them, and continued to feed the expectant mothers through pregnancy, and then both mothers and offspring through suckling until weaning (which in mice is around 20 days). The offspring themselves were fed on the radioactive lysine until they were 60 days of age. During their entire development, the young animals thus had a radioactively labelled material in their diet. So all their proteins would contain radioactive and not ordinary lysine. At 60 days of age (i.e. young adulthood) the diet was changed. The radioactive lysine was omitted and at various times afterwards animals were killed and the radioactivity present in the protein of their brains was determined. Lajtha obtained the following results:

Table 1

Days after diet change	Radioactivity in protein (disintegration/min/100 mg protein)**
0	1 680
30	492
60	143
150	81

** *Disintegrations per minute per* 100 *mg of protein. See Unit* 6, *section* 6.3.

* *This exercise is designed to relate to objectives* 2 (*ii*) *and* 3 (*i*).

Plot the data on the graph of Figure 1, expressing the radioactivity of the proteins at various times as a percentage of that at time 0.

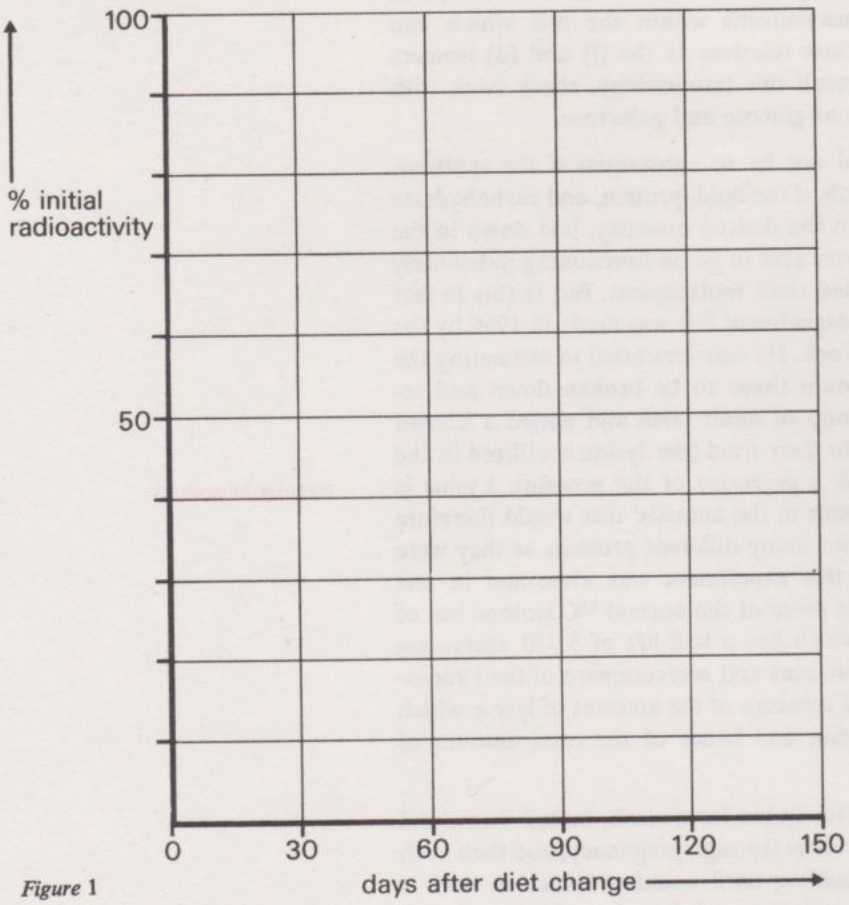


Figure 1

What do you conclude concerning the radioactivity in the protein?

There is an approximately *exponential* (see Unit 2) decline in the radioactivity of the proteins.

Which of the following interpretations do you regard as best fitting the data?

(1) The radioactive ^{14}C in the protein was decaying to a non-radioactive isotope.

(2) There was an exchange of lysine-containing proteins between the brain and elsewhere in the body.

(3) The protein of the brain was decomposing and the fragments were being removed from the brain but not replaced.

(4) The protein molecules of the brain were breaking down and being replaced by molecules of the same protein containing non-radioactive lysine.

To see the reason for the answer (which is given in the margin at the bottom of p. 12) consider the following points.

1 If the radioactive ^{14}C of the lysine in brain proteins was decaying to a non-radioactive isotope, then, while the amount of radioactive material per 100 mg of protein extracted from the brain would decrease, this would not mean a decrease in the amount of protein in the brain nor an alteration in the amount of lysine in brain proteins. But if radio-

active decay were responsible for the decrease in ^{14}C, then 50 per cent of the isotope ought to disintegrate in 5 770 years, which is the half-life of ^{14}C. As it is, the results show that $\frac{492}{1680} \times \frac{100}{1} = 29.2$ per cent of the isotope is left after 30 days—approximately 70 per cent disappears in 30 days. Clearly the radioactive decay of ^{14}C cannot by itself explain this rate of decrease.

2 The results could be explained as the outcome of an exchange between lysine-containing proteins in the brain and similar proteins elsewhere in the body. But if this occurred on a large scale then, as *all* the lysine-containing proteins synthesized up to the time the diet was changed could only have been made from lysine-containing ^{14}C, exchanging brain for body proteins would not alter the amount of labelled protein in the brain—assuming as much brain protein entered the body as body protein entered the brain.

Suppose an exchange between protein did in fact occur, then the only way by which there could be a drop in the amount of ^{14}C in the brain would be as a result of a far greater export of brain protein from the brain than import into it. And presumably this would mean that the animals' brains would shrink at a rate of 70 per cent in 30 days. So this explanation is unlikely.

For similar reasons, exchange of lysine, rather than whole proteins, between brain and body can be ruled out.

3 Suppose that, instead of an exchange of material between brain and body, the lysine or protein-containing lysine in the brain was decomposing and the fragments were being removed from the brain. This sort of process, if it happened, could explain the results—but while such a process might take place, it seems unlikely to happen on the scale required, simply because of the disorganization of cells which would be involved.

4 Finally Lajtha's results could be explained if either the ^{14}C-lysine or the proteins containing this lysine were being replaced by a non-radioactive lysine entering the body in the food. If this happened then we must accept the idea that there is a *turnover* of material in the body —a swapping over of molecules in body cells, or at least brain cells—with similar molecules entering the body from the world around.

It is not useful to talk of a time at which *all* the protein in the brain has been replaced. As you can see, the curve you have plotted from the data presented approaches zero asymptotically—even if you extrapolate it indefinitely, there is likely always to be some residual radioactivity left. A more meaningful term to calculate is the *half-life* of the protein: the time taken for 50 per cent of it to be replaced. Although the turnover rate of the many different types of protein in the brain will not be identical, it is possible to calculate, from your graph, an average half-life for the brain proteins.

What figure do you obtain?

It should be around 15–20 days. This and some other experiments show that more than 90 per cent of brain proteins have a half-life of between 13 and 20 days. It follows from such experiments that, even in the adult animal, each individual brain cell must be breaking down and resynthesizing some hundreds of protein molecules a minute.

Experiments of this type, which introduced the use of radioactive isotopes as a powerful tool of biochemical analysis, were pioneered in the 1920s by Hevesy in Copenhagen and continued with great distinction by Schoenheimer in America. They revealed that all body components are in a constant state of flux; that protein, lipid and nucleic acid molecules were constantly being renewed, the old molecules being broken down and new ones synthesized to take their place. Even the molecules of such stable and unreactive tissues as bone and cartilage, which used to be regarded as quite inert, were found to have quite a short life-expectancy. Throughout

the body, the molecule which survived for more than a few days without undergoing change was found to be the exception rather than the rule. During an average lifespan of seventy years, we have all exchanged many times over all the molecular components of every structure of which our bodies are made. The discovery of the prodigality of this constant flux of molecules revolutionized biochemical thinking. It became clear that one major function of the living cell was the constant re-creation of itself from material obtained from the external environment.

So before it can even begin to act on its external surroundings, the cell, or the living organism, has to provide the means whereby, first, it can protect itself against dissolution and destruction by the outside world, and second, it can continuously resynthesize its more complex parts from much simpler molecules. How does the cell set about achieving the large number of highly complex and specific chemical reactions necessary for the synthesis of the macromolecules?

Answer to question on p. 10.

The most probable interpretation of these results is the fourth one.

15.3 Enzymes

Study Comment

The synthesis and breakdown of substances inside living cells proceed by a series of stepwise chemical reactions, each of which is catalysed by an *enzyme*. Enzymes are protein molecules which function by forming intermediate complexes with the substances they react with (known as *substrates*). Enzyme reactions can be speeded up by *coenzymes*, and slowed down by *inhibitors*.

In this section, the major rules that govern catalysed reaction systems are reviewed and the most important characteristics of enzymes and the systems within which they operate are discussed (15.3).

Section 15.3.1 is a home experimental section, and 15.3.2 is a summary of the whole of 15.3.

You should be able to illustrate the general rules of enzyme action with particular examples, summarize the key features of enzyme kinetics, account for the specificity of enzymes in terms of the properties of the active centre, and summarize the general role of activators and inhibitors.

How are complex reactions achieved within the living cell? The types of chemical reaction you have studied so far have been carried out in test-tubes, flasks or beakers (the biologist calls such reactions *in vitro*, Latin for 'in glass', to contrast them with reactions occurring within the living organism or *in vivo*). To start them going, one frequently has to warm the reactants or alter their pH by adding acid or base. The cell can do none of these things. The chemicals of which it is composed begin to disintegrate if their temperature is raised too high or if their pH is shifted much from neutrality (indeed, as we shall see later in this Course, the cells maintain elaborate mechanisms to prevent the pH altering from its intracellular level of around 7.4). In addition, many *in vitro* chemical reactions result in the production of a mixture of products, whereas the feature that is all-important in cellular reactions is their specificity; that is, in the cell only one of a number of thermodynamically possible reactions involving a particular molecule will occur, and only one of a range of possible products will be generated.

specificity

In earlier Units, you have already come across a chemical phenomenon which enables reactions to occur more readily than they otherwise would do. Can you recall its name?

The phenomenon of catalysis (Unit 12). The secret of the cell's capacity to achieve the reactions required to maintain itself lies in the existence within the cell of a very large number of specific catalysts. These biological catalysts are collectively known as *enzymes*, and mention was made of them in Units 10 and 13.

enzyme

List the major rules which govern the properties of catalysed reaction systems.

If you do not recall these from Unit 12, look now at *The Chemistry of Life*, p. 88, where they are summarized from the biological point of view. Now read the section of *The Chemistry of Life* on enzyme properties (pp. 88–100).

Commentary

For the purposes of the present Course, you should be able to illustrate the general rules of enzyme action with particular examples, summarize the key features of enzyme kinetics, account for the specificity of enzymes in terms of the properties of the active centre, and summarize the general role of activators and inhibitors. You need not remember the enzyme classification suggested on pp. 98–100 of *The Chemistry of Life*, but we suggest you read this for the sake of completeness and background.

Glossary for pp. 88–100 of *The Chemistry of Life*

p. 88 *Raney Nickel*—a particular preparation of the metal, nickel, which is catalytically active.

p. 91 *streptococci*—plural of streptococcus, a particular group of bacteria.

p. 93 *spectrophotometer*—a device by which light of a specific wavelength can be passed through a sample in solution . The percentage of the light transmitted through the sample is a function of the amount of the sample present. You will understand its function more clearly when you come to use your own colorimeter (which is a more elementary version of a spectrophotometer) in section 15.3.1. Spectrophotometers and colorimeters can be used to obtain a measure of the amount of a substance, such as the product or substrate of an enzyme reaction, present at any given time. A spectrophotometer is used in the TV programmes of both Units 15 and 16.

Now do SAQs 1–6

15.3.1 Enzyme reactions: some practical exercises*

In these experiments, you are asked to investigate the characteristics of a particular enzyme reaction. The one chosen is that of the breakdown of starch by the enzyme *amylase*.

Recall, from Units 10 and 13, the composition of starch.

It consists of repeating linked chains of glucose residues. Amylase breaks down the starch, releasing glucose. You are provided with a solution of starch as *substrate* (that is, the substance on which the enzyme acts), and the source of the enzyme used in this experiment is your own saliva.

Salivary amylase is the enzyme responsible for the initial breakdown of starch during the digestion process. To detect the presence of starch, a colour reaction with iodine is used. A solution of starch, when mixed with one of iodine/potassium iodide, give a deep blue colour. The intensity of the colour depends on the amount of starch present. In this experiment, you will estimate the course of the enzyme reaction of the amylase with starch over time, and the effect of various treatments upon the enzyme.

salivary amylase

* *These exercises are designed to relate to objective* 5.

Apparatus required

1 Test-tubes
2 Plastic syringe
3 Colorimeter
4 Saucepans of hot water and ice or cold water
5 Thermometer
6 Stopwatch
7 pH paper
8 Meths burner

Chemicals required

1 Starch solution. Prepare by making a paste of 0.2 g of starch (provided) in 1 cm^3 water. Mix this paste with 100 cm^3 of boiling water in a beaker. Then boil for 1 minute. Cool to room temperature before use.

2 Solution of iodine in potassium iodide ('I/KI') containing 0.001 N I_2 and 2 per cent KI. This is provided.

3 Buffer tablets, pH 4.2, 7.0 and 9.2.

4 Distilled water, about 500 cm^3 (to be obtained from your local garage or chemist).

5 Salivary amylase. Obtain, for your stock solution of the enzyme, about 15 cm^3 of your own saliva in a small beaker. To do this, rinse your mouth out with water and spit out the rinse. Then swill a few cm^3 of water round in your mouth for several moments and collect the saliva produced in a beaker.

Outline of the investigation

The purpose of this set of experiments is to study a number of factors affecting the rate of an enzyme reaction. The rate of breakdown of starch by the enzyme amylase is measured under different conditions by incubating the starch with the enzymes for varying periods. The amount of starch remaining after the reaction is measured using the starch/iodide colour reaction.

Preliminary work

Set out your chemicals and apparatus. Recall from Unit 12 how to operate the colorimeter.

Before studying the enzyme, you will need to make sure that the starch/iodide reaction really does allow you to estimate the amount of starch present in the solution. To do this you should estimate the colour intensity produced in the reaction when varying quantities of starch solution are mixed with a standard amount of I/KI.

Take six test-tubes, and add reagents as shown in Table 2, p. 16.

Table 2

Test-tube No.	Water (cm^3)	Starch solution (cm^3)	I/KI (cm^3)
1	4.00	1.00	1.00
2	4.25	0.75	1.00
3	4.50	0.50	1.00
4	4.75	0.25	1.00
5	4.90	0.10	1.00
6	5.00	0.00	1.00

Read the colour intensity obtained in each of these tubes using the red filter in the colorimeter.

Plot a graph of colorimeter reading obtained against amount of starch added. This graph is now a *standard curve* for the starch/iodide reaction, and you should find that it is approximately (not quite) linear.

What information does this give you regarding the starch/iodide reaction?

That the colour developed is directly proportional to the amount of starch present. The more colour the more starch.

Note that, as your colorimeter is calibrated so that a reading of 100 forms your 'blank' value, the curve you obtain will be one in which the colorimeter reading *decreases* as colour intensity *increases*; i.e. the *higher* the colorimeter reading, the *less* starch is present. In the enzyme reaction, you will be measuring the disappearance of starch in the presence of the amylase. The higher the colorimeter reading, the less starch is present, and hence the more reaction products have been formed. Thus the colorimeter reading, R, is directly proportional to product formed P ($R \propto P$). A plot of colorimeter reading against time will then be of the same form as a plot of product against time, which is the measurement we wish to make. In the subsequent enzyme reactions you will therefore be able to measure the amount of starch present in the reaction mixture by determining the colour obtained in the starch/iodide reaction.

Experiment 1

The effect of amylase concentration on reaction rate

On p. 89 of *The Chemistry of Life* is the following passage:

> although the catalyst may participate in the reaction it catalyses, in the end it is recovered unchanged from the reaction mixture . . . a very tiny amount of catalyst can be used over and over again by a very large number of molecules of the reactant

If this is a true statement, and assuming the starch/saliva reaction is typical of other enzyme-catalysed systems, predict the time course of reaction between the following:

(a) 5 cm^3 of starch and 1 cm^3 of saliva

(b) 5 cm^3 of starch and 0.5 cm^3 of saliva

(c) 5 cm^3 of starch and 0.1 cm^3 of saliva

if all reactions are carried out under identical conditions of temperature and pH (look at Figure 8 (p. 92) of *The Chemistry of Life* to remind yourself of the general shape of enzymic reactions).

Make your predictions by drawing three curves in the graphs of Figure 2, one for each reaction mixture.

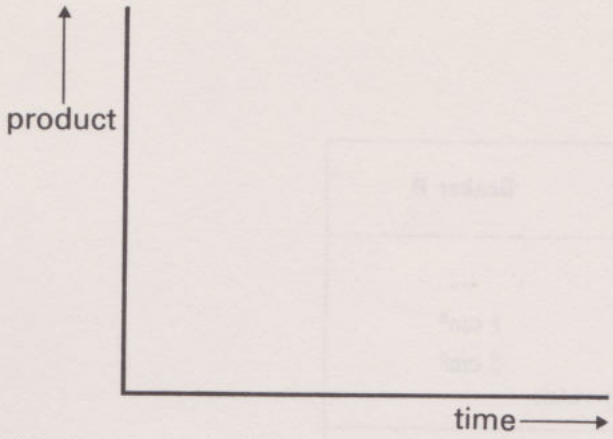
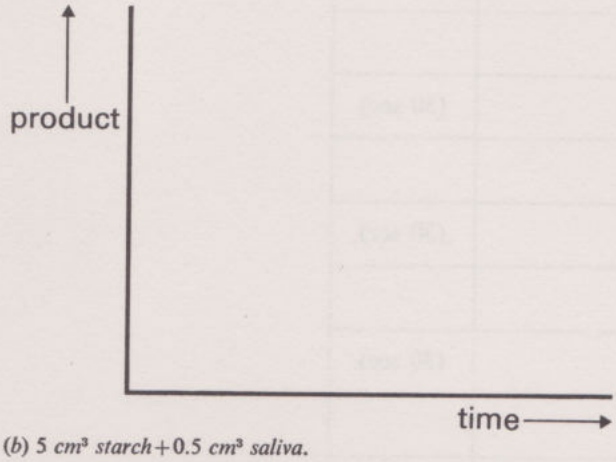
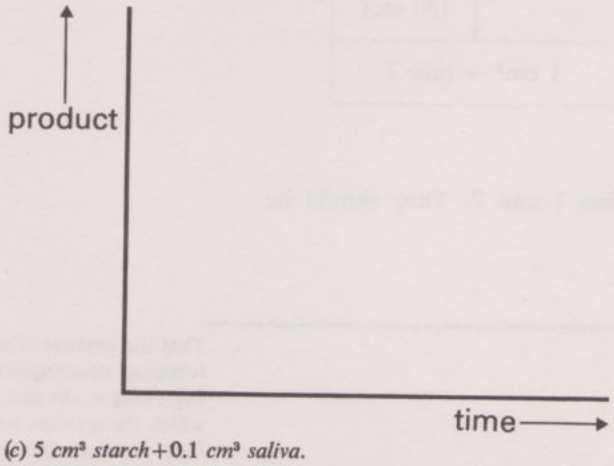


Figure 2 (*a*) *5cm³ starch+1 cm³ saliva.*



(*b*) *5 cm³ starch+0.5 cm³ saliva.*



(*c*) *5 cm³ starch+0.1 cm³ saliva.*

Having made your predictions, you can proceed to test them as follows. Prepare seven colorimeter tubes by adding 4 cm^3 of distilled water and 1 cm^3 of I/KI to each. Label them 1–7.

Now take two beakers, A and B. To A add 1 cm^3 of saliva. To B add 1 cm^3 of distilled water. Have ready your stopwatch, and a 1 cm^3 plastic syringe.

Add 5 cm^3 of starch solution to beaker B. Then add 5 cm^3 of starch solution to beaker A, and shake each beaker well. Take 1 cm^3 from beaker B and add to tube 1. After 30 seconds take 1 cm^3 from beaker A and add to tube 2. At 30 second intervals, take further portions of 1 cm^3

from A and add to tubes 2–6. After 2.5 minutes, that is, at the end of the experiment, take 1 cm^3 from beaker B and add to tube 7. The full procedure is set out in Table 3.

Table 3

	Beaker A	Beaker B
Saliva	1 cm^3	—
Water	—	1 cm^3
Starch	5 cm^3	5 cm^3
	start stopwatch	
	shake 30 sec	1 cm^3 → tube 1
	1 cm^3 → tube 2	
	(30 sec)	(30 sec)
	1 cm^3 → tube 3	
	(30 sec)	(30 sec)
	1 cm^3 → tube 4	
	(30 sec)	(30 sec)
	1 cm^3 → tube 5	
	(30 sec)	(30 sec)
	1 cm^3 → tube 6	1 cm^3 → tube 7

Compare the readings you obtain for tubes 1 and 7. They should be identical.

What can you conclude from this?

That the amount of starch present remained unchanged from the beginning to the end of the time during which the reaction was taking place, if there was no saliva present.

What is the purpose of including these two tubes in the experiment?

They form controls to show the extent of the spontaneous breakdown of starch.

Plot the colorimeter reading (which is, from your preliminary experiment, a direct measure of the amount of starch present, and hence also of product formed) in tubes 2–6 against the time at which the sample you read was removed from the enzyme mixture and added to the I/KI. Plot the time as the *x*-axis (as 0, 30, 60, 90, 120, 150, 180 seconds) and the colorimeter reading on the *y*-axis. This is called a *time-course*. What can you conclude from it?

Depending on the activity of your amylase, the time-course may look like curve (a), (b) or (c) of Figure 3.

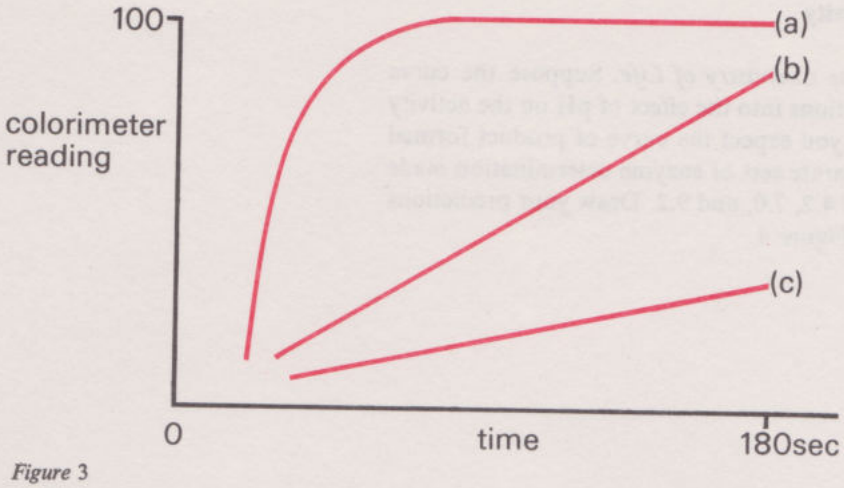


Figure 3

To investigate the effects of dilution on the enzyme reaction, if you get a curve similar to (a), repeat the experiment with smaller amounts of enzyme in beaker A (you do not need the control beaker B in this experiment). If the curve is similar to (b) or (c), you should try more enzyme as well as less. When we did the experiment, we got good curves with 1.25, 1.0, 0.75 and 0.5 cm^3 of enzyme. Yours may well be different depending on the activity of your amylase and the way in which you washed out your mouth. Plot all your curves on the same graph.

On the basis of your experimental results, see if you can answer the following questions. (Note: there are no formal answers given for these questions and for those following in the remainder of this section.)

Questions

1 Does the rate of the reaction alter when the maximum enzyme concentration you used is lowered?

2 In general, what is the relation between rate of reaction and concentration?

Do the curves you obtain match the predictions you drew on Figure 2?

3 To what extent is it true that increasing enzyme concentration increases the rate of reaction?

4 If you think, as a result of your experiment, that the statement on p. 89 of *The Chemistry of Life* is true, would you be prepared to say that it was always true, for all concentrations of enzyme and substrate, or are there limitations to it?

Test the residual solution in the tubes in which the enzyme-starch reaction has occurred, and in those in which distilled water replaced the enzyme, by dipping a 'clinistick' into them. Clinisticks contain a test preparation which turns blue in the presence of glucose solution.

5 In which tubes do the clinisticks turn blue?

6 Which tubes contain glucose?

7 How can you account for the presence of glucose in these tubes?

8 How can you account for its absence in the others?

The effects of pH on amylase activity

Look at Figure 9 on p. 93 of *The Chemistry of Life*. Suppose the curve resulted from a series of investigations into the effect of pH on the activity of salivary amylase. How would you expect the curve of product formed against time to look for three separate sets of enzyme determination made at different pHs, for example, pH 4.2, 7.0, and 9.2. Draw your predictions as three curves on the graph of Figure 4.

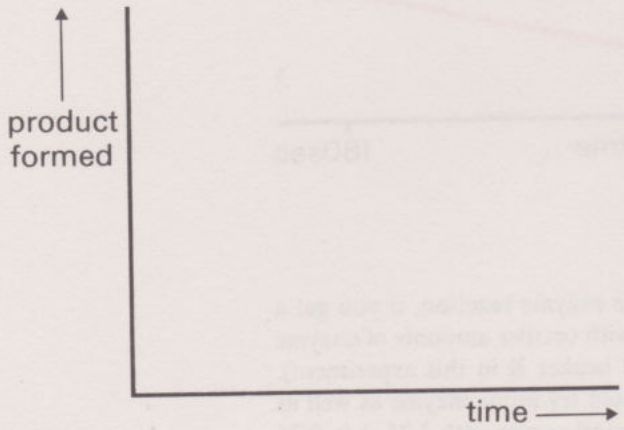


Figure 4

You can test your predictions by experiment.

Prepare three solutions, of pH 4.2, 7.0 and 9.2, by dissolving the buffer pellets provided each in 100 cm^3 of distilled water.

Choose an enzyme concentration, from your graph of Experiment 1, which will result in about 50 per cent of the starch being broken down in 2.5 minutes (let us call this amount of enzyme solution x—it was about 0.75 cm^3 in our experiments). If you are not sure how to do this, look at the graphs you obtained from Experiment 1. Recall that colorimeter reading is proportional to product formed. A colorimeter reading of 100 is equivalent to zero starch and 100 per cent product; a colorimeter reading of 50 is equivalent to 50 per cent starch and 50 per cent product.

Set up four beakers A, B. C, D, as follows:

Table 4

Beaker	Buffer solution (cm^3)	pH	Distilled water (cm^3)	Starch (cm^3)
A	1.0	4.2	0	5.0
B	1.0	7.0	0	5.0
C	1.0	9.2	0	5.0
D	0	—	1.0	5.0

Prepare four colorimeter tubes, each containing 1 cm^3 I/KI and 4 cm^3 water, labelled 1–4. Have your stopwatch ready, and add x cm^3 of salivary amylase to each of the beakers A–D. Shake. 1 minute after the addition of the enzyme, withdraw 1 cm^3 from each beaker and add to one of the iodine/potassium iodide containing tubes. Read tubes 1–4 in the colorimeter.

Plot a graph of colorimeter reading 1 minute after the start of the enzyme incubation (y-axis) against pH of the incubation mixture (x-axis). From the curve you get, estimate the pH of salivary amylase in the absence of buffer.

Questions

9 What shape does your graph have? Does the curve you obtain match the predictions you drew on Figure 4?

10 What can you deduce about the effects of pH in salivary amylase activity?

11 What is the optimum pH for the amylase?

Experiment 3

The effect of heat or acid on amylase activity

From your graph of Experiment 1, choose an amount of amylase sufficient to break down all the starch in the mixture within 2.5 minutes. Let us call this amount x cm³ (in our experiments, it was 1.2 cm³).

Set up six colorimeter tubes, 1–6, each containing 4 cm³ distilled water and 1 cm³ of I/KI. Have ready your stopwatch, and set up three beakers, A, B and C, containing 5 cm³ of starch solution. Add three drops of 2M HCl to B.

Put 1.5 cm³ of the salivary amylase into a separate tube and boil the amylase gently for 1 minute by holding the tube in the meths burner. Allow the amylase to cool to room temperature again before proceeding.

Now add x cm³ of fresh saliva to beakers A and B, and x cm³ of *boiled* saliva to beaker C. Shake, start the stopwatch, and immediately transfer 1 cm³ from each of beakers A, B and C into tubes 1, 2 and 3.

After 2.5 minutes, withdraw a second portion of 1 cm³ from each of beakers A, B and C and add to tubes 4, 5 and 6.

Read tubes 1–6 in the colorimeter and test the remaining contents of beakers A, B and C with clinisticks.

What readings do you obtain? Draw them up on a table as follows:

Table 5

Reading	A (standard)	B (acid)	C (heat)
Time 0 min. Time 2.5 min.			
Clinistick test (+ or −)			

Question

12 What do you conclude about the effects of acid and heat upon the salivary amylase, and how do you explain your results?

Experiment 4 (*optional, depending on time available*)

Effect of temperature on amylase activity

If you have time, do the following additional experiment. Re-read *The Chemistry of Life*, p. 92, on the effect of heat on enzyme reactions. Examine Figure 8. The statement is made that:

> if we raise the temperature . . . not only does the initial reaction velocity increase . . . but the enzyme is also less stable and inactivated sooner.

On what grounds is the statement concerning reaction velocity based?

On the rules governing the velocity of catalysed reactions (refer back to Units 10 and 12 if you are uncertain on this).

On what grounds is the statement concerning reaction rate based?

On the fact of the thermal instability and ease of denaturation of protein (Units 13, 14).

So far, you have carried out the amylase experiments at room temperature. Predict the effect of a higher and a lower temperature on the reaction. Draw your predictions as three curves (room temperature, 60° C and 5° C) on Figure 5.

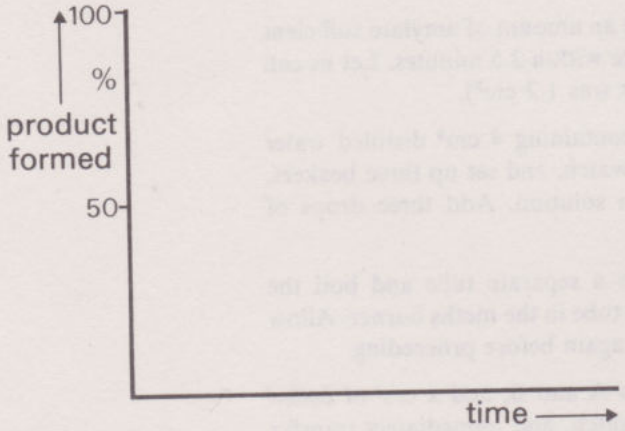


Figure 5

Now carry out an experiment to test your predictions.

Take a saucepan of hot (about 60° C) water or cold (as cold as you can get it, preferably iced) water. Put a beaker containing 5 cm^3 of starch in the saucepan. Leave it for about five minutes to come to the temperature of the water. Meanwhile set up six tubes containing 4 cm^3 water and I/KI as before.

Choose a concentration of amylase, from Experiment 1, sufficient to break down about 50 per cent of the starch in 2.5 minutes at room temperature (it was about 0.75 cm^3 in our experiments). Have ready your stopwatch, add the salivary amylase to the starch, and withdraw 1 cm^3 samples into the iodine potassium iodide at 30 second intervals as before. Read the tubes in the colorimeter and plot a time-course of the starch-amylase reaction as in Experiment 2.

Repeat the experiment using both hot and cold water to adjust the temperature of the reaction (such a procedure is called *incubation*; your saucepan is an *incubation bath*). Plot all the results on the same graph as a series of separate curves, indicating for each curve the temperature at which the readings were performed. You can note the room temperature and include the curve you obtained in Experiment 1 as one of these.

Repeat as many other temperatures as you see fit. A sensible choice would be 20° C, 30° C, 35° C, 40° C and 50° C, though you could try others, including hotter ones yet.

Questions

13 Which temperature gives maximum breakdown of starch after 30 seconds?

14 Which gives maximum breakdown after 1 minute?

15 Which gives maximum breakdown after 1.5 minutes?

16 What do you conclude concerning the effect of temperature on enzyme reactions?

15.3.2 Summary of sections 15.2 and 15.3

The chemicals of which the cell is composed are in a state of constant flux. Although, in the adult, the total amount of protein, lipid and nucleic acid in any organ is more or less constant, the individual molecules are constantly being broken down into simpler molecules and resynthesized from simple molecules once more.

The reactions of synthesis and of breakdown are often complex and cannot occur spontaneously under the conditions of pH, temperature, etc. found within the cell. They must be catalysed. The catalysts of biological systems are known as enzymes. Enzymes have a set of properties as catalysts in part dependent upon the fact that they are proteins. Each enzyme generally catalyses only a single reaction. The velocity of enzyme-catalysed reactions can be modified sharply by pH, temperature and the presence of substances which either activate or inhibit the enzyme.

Self-Assessment Questions 1–7 *relate to section* 15.3 *of this Unit. You may like to attempt them now, before proceeding further.*

15.4 Cell Energetics and the Role of ATP

Study Comment

Some reactions within the cell are exothermic, some endothermic. In general, synthetic reactions, like the production of protein from amino acids, are endothermic. To 'drive' the endothermic reactions, the molecules involved in the synthesis are activated, often by phosphorylating (adding phosphate to) them. This phosphorylation is performed at the expense of the substance *adenosine triphosphate* (ATP for short). In its turn, ATP is synthesized from *adenosine diphosphate* (ADP) and phosphate during exothermic reactions such as the oxidation of glucose (15.4).

The role of ATP in the economy of cells is discussed and should be understood thoroughly because the cellular use of ATP is ubiquitous. This is the central theme of this section of the Unit.

The properties of the enzymes present in the cell account for the specificity of the reactions carried out in the synthesis and breakdown of the molecules of which the cell is composed. They do not, however, solve the problem of cell *energetics*. Many of the reactions carried out within the cell are *endothermic*. (If you do not recall the meaning of this term, refer back to Unit 11). The synthesis of a protein from its constituent amino acids is a thermodynamically unfavourable reaction. In order for the cell to perform this type of reaction, not only must it have the complement of specific enzymes necessary for the particular cellular reaction sequence to occur, but in addition the endothermic reaction must be provided with an energy input. This input cannot be in the form of heating the reactants, or passing an electric discharge through them, or any one of the other possible inputs available in a non-biological system, for such a procedure would result in cell destruction or death. Instead, what happens is that, running in parallel with the synthetic endothermic cell reactions, a series of exothermic reactions take place. The endothermic and exothermic reactions are linked, in a way we will discuss shortly, thus producing a set of reactions whose net energy change is either zero or negative. So the synthetic reactions are made thermodynamically feasible for the cell.

cell energetics

From your general knowledge, you perhaps know the ultimate source of energy for biological systems. What is it?

The Sun.

Green plants operate a reaction whereby they can tap the solar energy which reaches the Earth's surface. This light energy is used for synthesizing sugars such as glucose from the simpler molecules of carbon dioxide (we will discuss this mechanism, that of *photosynthesis*, later in this Unit). Plants can then break down the glucose and polysaccharides to release the energy once more when they require it. Animals have no primary source of this sort, and must rely on being able to find a ready-made supply of potted energy in the form of sugars or related substances. Some (*herbivores*) achieve this by devouring the plants and using the materials that have already been synthesized. Other animals (*carnivores*) devour the animals that devour the plants, and the cycle of mutual interdependence is completed when the plants themselves make use of some waste-products that the animals release, such as carbon dioxide (see Unit 20).

herbivores
carnivores

Throughout the whole cycle, the law of conservation of energy holds. Many experiments have been done in which animals, or human volunteers, are kept for several days in closed boxes or rooms. The energy provided for

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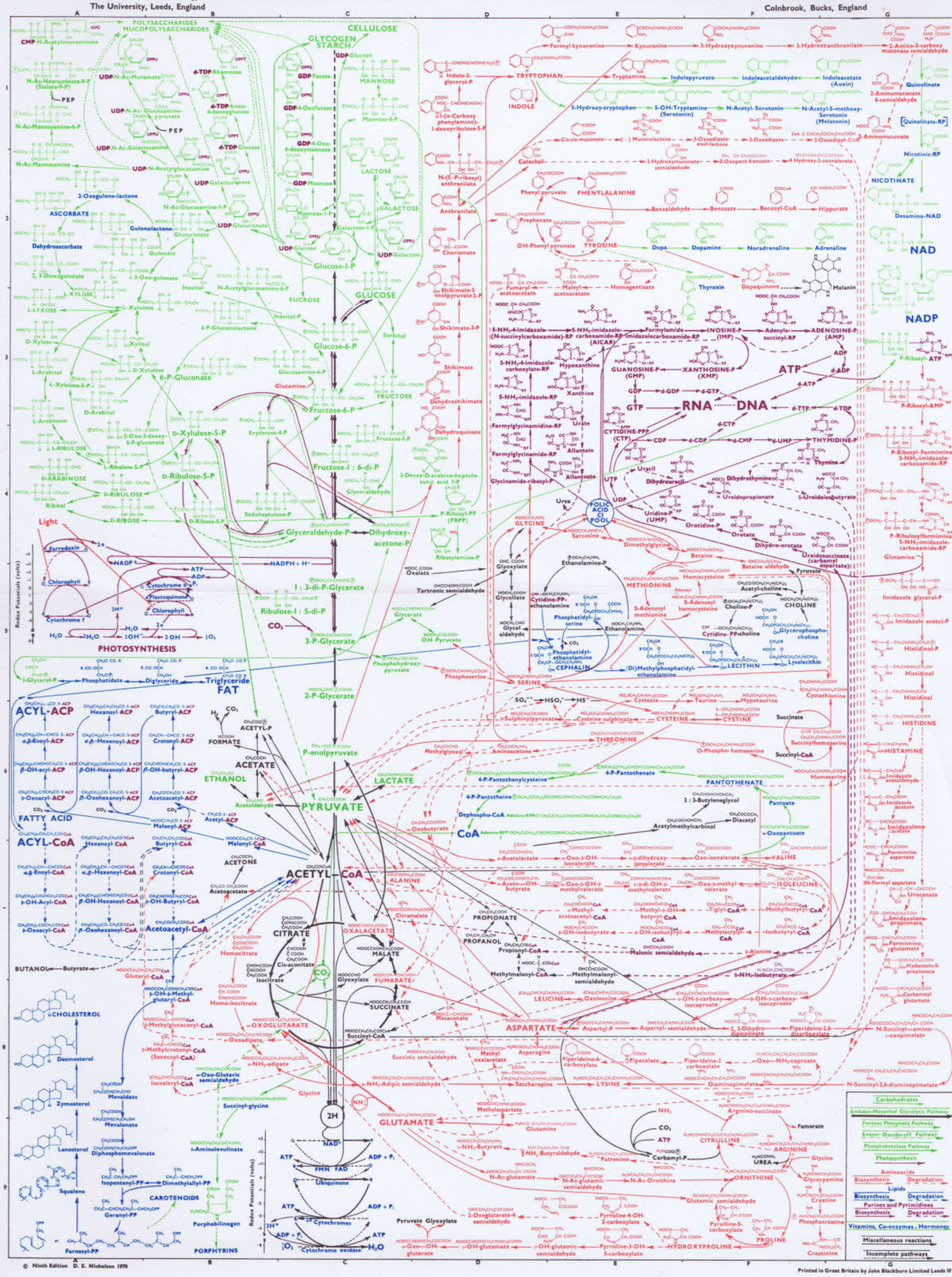

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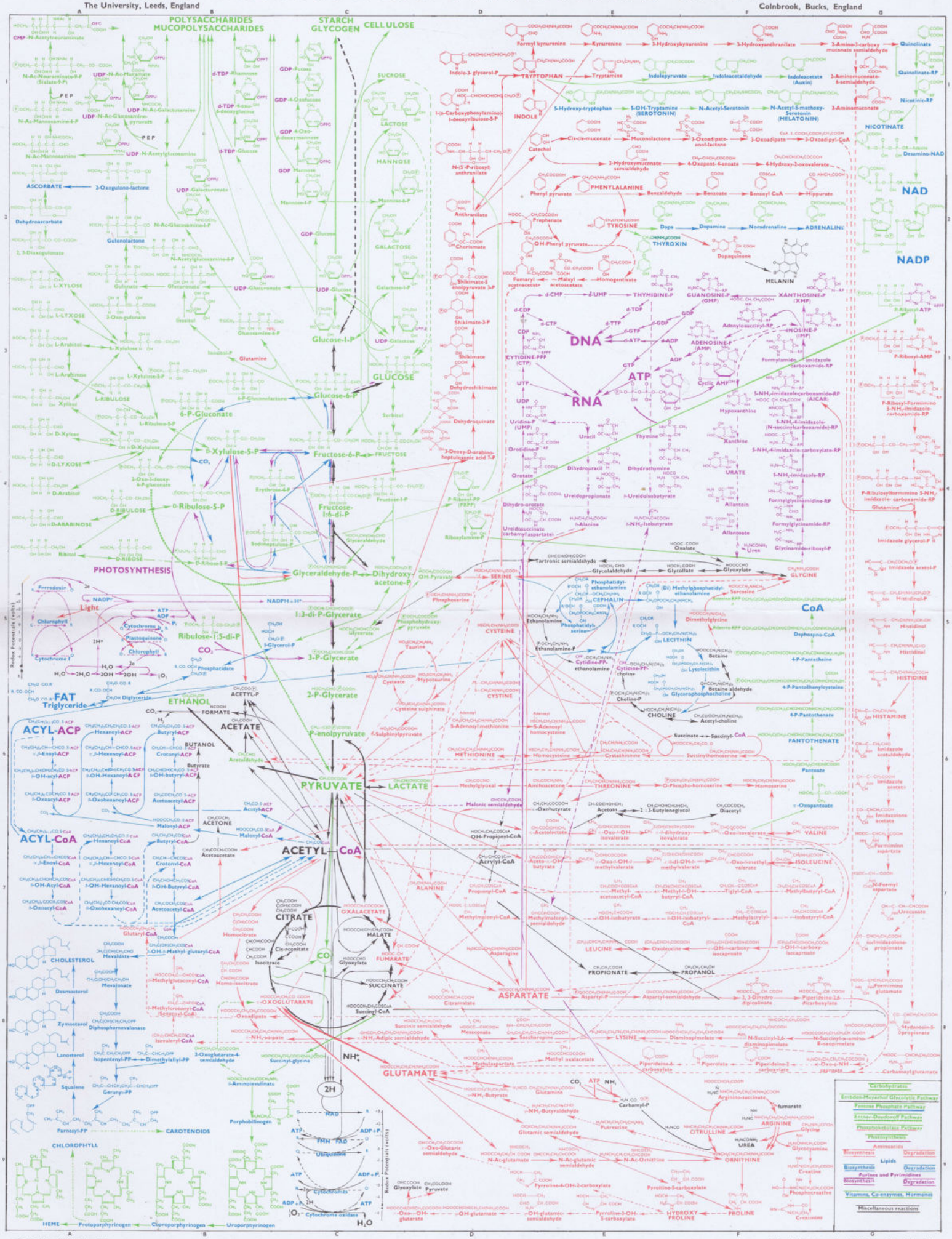

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them in the form of food is compared with that utilized—as heat output, carbon dioxide production, elimination of nitrogen-containing compounds such as amino acids and urea in faeces and urine, and work done (for example, by animals in treadmills or by humans riding stationary bicycles). Within the closest obtainable experimental limit, both men and animals obey the law of conservation of energy, input and output being exactly balanced.

At the cellular level, the principal exothermic reaction in both animals and plants is the *oxidation* of glucose; glucose derived in the case of plants by the 'fixing' of CO_2 during photosynthesis, and in the case of animals by eating the plants or other animals.

oxidation of glucose

You will recall the definition of oxidation from Unit 8. Write the equation for the oxidation of glucose ($C_6H_{12}O_6$) by molecular oxygen, to carbon dioxide and water.

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$

This is an energy-yielding reaction. If you were to burn glucose in an atmosphere of oxygen in an appropriate vessel, you would release 2 850kJ per mole of glucose consumed. Within the cell, endothermic reactions occur by a process which couples oxidation of glucose to the endothermic reaction sequences. This coupling procedure, however, is an elaborate one. The cell performs the glucose oxidation reaction, but it does so in a rather roundabout manner. It breaks the glucose down by a series of reactions, each time releasing a small amount of energy only. The release of such a large amount of energy in one lump, so to speak, would be too much for the cell to cope with; much of the energy would be dissipated as heat, quite likely destroying the cell in the process. What the cell has been able to achieve is the controlled stepwise breakdown of glucose to provide a steady source of energy. Glucose is converted to CO_2 by a process involving nearly thirty different steps, each enzymically catalysed, in no one of which more than a small amount of energy is released. Thus the cell obtains its energy in the form of small 'packets' which can be conserved and used systematically.

The reactions involved in the oxidation of glucose within the cell form a complex sequence and it would be hard for them to be coupled directly to synthetic reactions. In fact, a far more ingenious system occurs within the cell and seems to be of universal biological significance. To see the details of this mechanism, turn to *The Chemistry of Life* and read pp. 82–85.

Commentary

The role of ATP, which is discussed there, needs the following further comment. The classification of a molecule like ATP as 'energy-rich', compared with AMP, which is 'energy-poor', must be seen as convenient biochemical shorthand. An energy-rich compound in this special, biochemical sense is one whose synthesis is the immediate consequence of energy-yielding reactions such as glucose oxidation, and whose breakdown to an energy-poor compound again occurs during synthetic reactions such as protein synthesis. One can speak, loosely, of the energy released during glucose oxidation being stored as ATP and subsequently being used to drive the synthetic reactions. The difference between ATP and an energy-poor substance like AMP can be seen if we compare the energy released during the hydrolysis of a mole of ATP to ADP, inorganic phosphate and a hydrogen ion—about 33.5 kJ mol^{-1} at pH 7.4—with that released during the hydrolysis of 1 mole of AMP to adenosine, inorganic phosphate and a hydrogen ion, which is about 15 kJ mol^{-1}. It is in this sense that the biochemist refers to energy-rich molecules, and the shorthand terminology used to indicate them is the 'squiggle' notation '~' introduced in

ATP

AMP

ADP

The Chemistry of Life. It should be made clear, though, that the ~ does not imply that the energy is somehow 'locked up' in the particular bond broken or formed when the reaction

$$ATP + H_2O \rightleftharpoons ADP + \textcircled{P}^- + H^+$$

occurs.

(Note that we have written the reaction more fully here than in *The Chemistry of Life* to show the role of the hydrogen ion released during the hydrolysis.)

Although biochemists find the 'energy-rich' concept a useful one, it is disliked by some chemical thermodynamicists because it seems to imply this 'locking up'. We shall use the terminology for convenience in these two Units, but you should be aware that in doing so we are to some extent masking a scientific controversy between different disciplines which is as yet unresolved.

Note that in *The Chemistry of Life* the text refers to energy-rich molecules in the sense discussed here. It also refers to a quantity called 'free energy'. This term is related to, though it is not the same as, the term 'enthalpy' introduced in Unit 11. In this Course it has not been possible to introduce the concept of free energy, nor is it necessary to understand it to obtain an adequate qualitative picture of the role of ATP. Another difference in terminology is the use in *The Chemistry of Life* of the terms 'endergonic' and 'exergonic'; these are closely related to the terms 'endothermic' and 'exothermic' used in this Unit and throughout the Course. Again, the precise distinction does not concern us here.

The striking thing about the cellular use of ATP is the ubiquity with which this mechanism appears in Nature. THIS IS THE CENTRAL POINT OF THIS SECTION OF THE UNIT. In all living systems that have been examined, whatever the reaction sequence that is used as the principal exothermic system—and not all organisms use glucose—the reaction to which the system is coupled is the synthesis of ATP.

In the cell, the reactions in which the energy-rich ATP is used, in its turn, to drive synthetic reactions do not occur by the simple hydrolysis of ATP. This would result in its energy being released in a way 'wasteful' to the cell, by dissipation as heat. Instead, the reaction frequently proceeds by the transferrence of the terminal phosphate of ATP to some other molecule that is a direct intermediate in the synthetic reaction; this molecule is thus *phosphorylated* and so able to participate in reactions which would otherwise be unfavourable. You will see examples of this use of ATP both later in this Unit, and in Units 16 and 17.

phosphorylation

You should now be able to summarize the relationships between complex and simple molecules and the roles of ATP and enzymes, in synthesis and degradation within the cell. Try to draw a simple 'flow diagram' of these relationships, and then compare yours with that in Figure 6 (yours may be better than ours, of course).

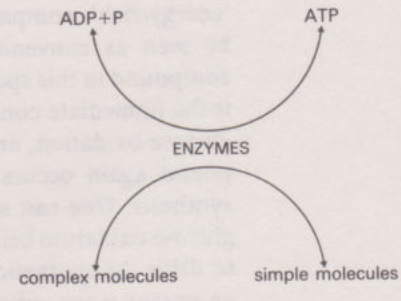


Figure 6

15.5 Cell Metabolism

Study Comment

A number of ways in which cellular reactions can be unravelled are described. You should read these descriptions carefully, not attempting to memorize them, but relating the techniques to their limitations, which are outlined in section 15.5.2.

metabolic pathways

There is more than one way of classifying *metabolic pathways*, the reaction sequences involved in the breakdown and synthesis of cell components. In the older textbooks, the division is normally made between pathways for the breakdown of already existing substances to simpler compounds—for example, the breakdown of protein to amino acids and their subsequent oxidation—and the reverse process, that of the synthesis of complex from simple substances, for instance, the synthesis of proteins from amino acids. The breaking-down reactions are called *catabolic*, the synthetic reactions are *anabolic*, and the sum of all the catabolic and anabolic reactions occurring at any time represent the total of the cell's *metabolism*. You can get some idea of the complexity of these reactions by looking (do *not* attempt to memorize!) at the 'metabolic pathways' chart of Figure 7, facing p. 24.

catabolic reactions
anabolic reactions

energy-yielding and energy-using reactions

Another way of looking at the same reactions is to divide them into *energy-yielding* and *energy-using* reactions. In general, energy-yielding reactions are catabolic, and energy-using ones are anabolic. But not all catabolic reactions yield energy. Many complex catabolic reactions within the cell actually require energy, but a cell performs them nonetheless, and in so doing rids itself of waste or toxic substances. We shall look first at the reactions by which the cell generates ATP, and leave consideration of the way in which it uses this ATP until Unit 16.

15.5.1 The study of cell metabolism

Before doing so, however, it seems proper to spend a little time considering operationally, how one can set about making experiments to investigate such reactions: individual cells are very small and the reactions that occur within them proceed very rapidly, involving the activity of very many enzymes. In investigating reactions concerned with the transformation of one substance into another within the cell (known in general as *metabolic processes*), we should be conscious of the techniques available for their study. For many purposes, instruments in biology need to be used at the limits of their resolution, just as was the equipment of Galileo for the formulation of his law of falling bodies, which you followed in Unit 1.

To see how such metabolic experiments can be made, turn now to *The Chemistry of Life* and read pp. 101–109, on pathways of metabolism; then try *SAQ* 7. Section 15.5.2 is a summary of those pages in *The Chemistry of Life*. If you are short of time, read only the summary, and do not attempt to read the prescribed text. Otherwise, read *The Chemistry of Life* first, and then make your own summary of the types of technique and analysis described there. Finally, compare your summary with that of section 15.5.2.

15.5.2 Techniques in cell metabolism: a summary

Faced with the problem of identifying the substrates, intermediates, products and enzymes involved in long and often complex reaction pathways, the scientist can avail himself of two types of techniques.

(a) Techniques for interfering with or 'marking' the chemicals of the reaction sequence. These include the use of *inhibitors*, and of *radioactive isotopes*.

(b) Techniques for 'simplifying' the organisms being studied, by isolating for study particular *tissues*, *organs*, *cells*, *sub-cellular fractions* or *enzymes*.

Each type of approach has its limitations; each is related to the others through the concept of *hierarchy*; an isolated enzyme is *part of* a more complex system, the metabolic pathway, which is *part of* the overall cellular economy, which is *part of* the properties of the whole cell, which is part of the behaviour of tissues, organs and organisms. To study any of these by simplifying the system under examination gives an answer, but only a partial answer, to the question of the properties of the whole system. (We return again to this point at the end of Unit 16.)

In order to study the kinetics and dynamics of the living system, it is necessary to interfere with it and to interrupt its 'normal' behaviour. This interference itself is such as to *alter* the behaviour of the system. At worst, such studies may lead to the type of artefact discussed in Unit 14. At best, they can provide only successive approximations to the full statement of the behaviour of the cell. You will find in subsequent Units that analogous problems also occur, for example, in physics when the motion and position of electrons is studied. In physics, these uncertainties of observation and study have been formalized under the name of the 'Uncertainty Principle' (Unit 29).

15.6 The Sources of Cellular Energy

Study Comment

The major energy-yielding (ATP-synthesizing) pathway of the cell, that of glucose oxidation, is described, with an exercise on experiments concerning glucose breakdown during muscle contraction. Both glycolysis and the Krebs cycle are discussed in outline.

Do not attempt to remember the details of the experiments outlined in section 15.6.1, but you should understand how the conclusions are reached from the data given. The exercise in this section is designed to help you understand how experimental evidence is evaluated.

The experiment in section 15.6.2 is included to practise the same sort of skill, but is this time on data that you yourself collect.

Glucose breakdown and the Krebs cycle are outlined and discussed in section 15.6.3. Do not attempt to memorize details of these processes or the name of individual enzymes. You should, however, realize that the reaction sequences described are universal—occurring in all living organisms so far studied. You should also understand the importance of the stepwise degradation of the energy-yielding substrates involved, and the cyclic nature of the reaction sequences.

The substrates whose breakdown is exothermic and can be coupled to the synthesis of ATP arrive at the body as food, for all organisms except green plants. In many animals, the food reaches the cell only after it has already been broken down into relatively small molecules which the cell can easily absorb and handle. This process of breaking down is called digestion, and its details are well known. In man, food is broken down in the mouth, stomach, and intestine by a series of enzymes: polysaccharides by amylases in the saliva and pancreatic juice; proteins by pepsin in the stomach and trypsin and chymotrypsin in the intestine; and fats by lipases from the pancreas.

The resulting mixture of sugars, amino acids, and fatty acids is absorbed through the intestinal wall and in due course enters the blood stream. In the well-fed individual, every cell is thus kept steadily supplied with its food requirements in the form of low-molecular-weight substances dissolved in the circulating blood (see Unit 18).

Under normal circumstances, the bulk of the cell's energy requirements are met by the breakdown of sugars, although both fatty acids and amino acids can also be broken down and the energy released used to synthesize ATP. The situation in the green plant differs from that in the animal, *only* in that the plant manufactures its own glucose by photosynthesis prior to using it as a foodstuff. We shall discuss the mechanism of photosynthesis in section 15.8. Here, we turn first to the mechanisms of glucose breakdown.

For the reasons outlined in section 15.2 above, the breakdown of glucose must be conducted in a stepwise fashion in the cell. Why?

Because otherwise the possibility of linking individual reactions of the sequence to ATP production would be lost. Energy released during

oxidation would not be conserved but would be released in an uncontrolled way that might be damaging to the cell. In fact, the reaction sequence is a long and complex one, involving nearly 30 different steps. We are here going to set out the metabolic pathway of glucose oxidation in some detail, for three reasons. First, it is in many ways typical of the multitude of other cellular metabolic pathways shown in Figure 7. Second, it is by far and away the most important pathway involved in the generation of ATP for subsequent use, and in this Unit we are very concerned with discussing the workings of the cell in terms of *energy traffic* by way of ATP. Third, when in Unit 16 we turn to the topic of the *control* of cell metabolism, we shall once again use the same metabolic pathway to illustrate the problem of control and information flow within the cell. Much of the evidence concerning the sequence of reactions is based on studies in muscle and yeast. Before turning to examine the reaction sequence in detail, and in order to give you a 'feel' for the sorts of experiments and arguments used during its elucidation, do the exercise of section 15.6.1 and the experiment of section 15.6.2.

15.6.1 Experiments on glucose breakdown in muscle

Glucose may enter the animal cell either directly, from the circulatory blood stream, or be present within certain cells of the body, in its storage form as glycogen (Unit 14). From whatever source it is derived, glucose is broken down in two stages. In the first, the 6-carbon glucose molecule is split in half into two 3-carbon fragments each of which is oxidized to pyruvic acid ($CH_3COCOOH$), releasing only a little energy in the process. The first half of glucose breakdown is called *glycolysis*. In the second stage, the pyruvic acid is completely oxidized to carbon dioxide and water by way of a series of acidic intermediates. This stage releases a great deal of energy, and requires oxygen. It is the *oxidative* phase.

When a situation arises in which the cells need a great deal of energy very quickly, it may happen that the demand for oxygen is so great it outruns the supply available to the cell. (You will see in section 15.7 how oxygen gets to the cell.) When this happens, the cell falls back on operating the glycolytic half of glucose breakdown without the oxidative half. The energy yield from this is smaller, but it does mean that the cell has a second line of defence should its oxygen supplies fail it. This glucose breakdown in the absence of oxygen is called *anaerobic glycolysis*. The pyruvic acid produced is reduced to lactic acid ($CH_3CHOHCOOH$) and then disposed of by release into the blood stream. Anaerobic glycolysis provides much of the energy for any sustained bout of violent muscular exercise—a hundred yards sprint, for example—and, indeed, after such exercise it is possible to measure large increases in the level of lactic acid in the athlete's blood stream.

anaerobic glycolysis

Before looking at the reaction sequence in any detail, we want you to consider the types of experiment, more complex than those you can make at home, which have led to our current conception of the mechanism of glycolysis. To do this, we describe below some of the historic experiments made in the elucidation of the pathway of glycolysis in muscle. Note that the object of doing this is *not* that you should remember each experiment in detail, but that you should develop a 'feel' for the evaluation of scientific evidence.

Consider each piece of experimental evidence in turn. In each case we suggest some possible interpretations of the observations. Some of the interpretations are obviously incorrect, while some go beyond what can legitimately be deduced from the evidence presented. (Note: you should not regard any interpretation as 'correct' in some absolute sense, but merely as best fitting the available data.) Pick out and note what seems to you

to be the most appropriate interpretation before going on to the next piece of evidence. The legitimate interpretations, and commentary on the alternatives, will be found on pp. 33–35.*

Experimental evidence I

Fletcher and Hopkins in 1907, working with an isolated frog-muscle preparation, showed that:

1 Muscle can contract in the absence of oxygen.

2 Lactic acid is produced when muscle contracts. In the absence of oxygen it accumulates until the muscle is 'fatigued' and will contract no more. No further lactic acid is then produced.

3 On return to oxygen, the muscle can be made to contract again and the lactic acid disappears.

4 Less lactic acid is formed in a contracting muscle with access to oxygen than in one contracting in anaerobic conditions.

Possible interpretations of I

(a) Muscles need oxygen for contraction.

(b) Lactic acid is produced only when muscles contract anaerobically.

(c) When oxygen is lacking, lactic acid is produced faster than it can be removed.

(d) Lactic acid is oxidized in aerobic conditions.

(e) Lactic acid causes muscle contraction.

(f) No unequivocal interpretation is possible.

Experimental evidence II

1 By 1920, it was known that the muscles of the frog and other vertebrates contained glucose stored in the form of glycogen (Unit 14) and that the amount of the glycogen present was reduced after a series of contractions.

2 By 1927, it had been shown by Meyerhof and others that, in a muscle contracting in anaerobic conditions, the amount of lactic acid produced was directly proportional to the amount of glycogen that disappeared from the tissue. (To be exact, two molecules of lactic acid were produced for each molecule of glycogen disappearing.)

There was also a proportionality between the pull developed by muscle and the weight of lactic acid produced.

Possible interpretations of II

(a) Lactic acid in contracting muscle comes from glycogen breakdown.

(b) Glycogen supplies the energy for muscle contraction.

(c) Reactions in which glycogen disappears and lactic acid accumulates are coupled in some way with muscular contraction.

(d) No unequivocal interpretation is possible.

* *This exercise is designed to relate to objectives* 2 (*ii*) and 3 (*i*).

Experimental evidence III

1 In 1927, a substance, creatine phosphate, was isolated from muscle and found to produce considerable heat when hydrolyzed to creatine and phosphoric acid.

2 Lundsgaard in 1930 showed that muscle would contract anaerobically *without* lactic acid accumulating if he first 'poisoned' the muscle with iodoacetic acid (an enzyme inhibitor). The contraction in these conditions was accompanied by the breakdown of creatine phosphate (CP) to creatine (C) and phosphate (P) and when this substance was used up, contractions ceased.

3 In 'unpoisoned' muscle, CP was shown to be broken down during activity and resynthesized at rest.

Possible interpretations of III

(a) When iodoacetic acid is present during muscle contraction, glycogen is not broken down to lactic acid.

(b) Creatine phosphate is responsible for muscle contraction.

(c) The conversion of glycogen to lactic acid is, in some way, responsible for the resynthesis of CP.

(d) No unequivocal interpretation is possible.

Experimental evidence IV

1 By 1929, the presence of adenosine triphosphate (ATP) in muscle had been demonstrated. ATP was known to be 'energy-rich', as described in section 15.4 above.

2 In 1934, there was isolated from muscle an enzyme which catalysed the reversible reaction $ATP + C \rightleftharpoons ADP + CP$.

3 When this enzyme was prevented from working by an inhibitor, the number of contractions that could occur before the muscle became 'fatigued' and ceased to function was very much reduced. Under these conditions, the CP content of the muscle remained the same, but the ATP concentration went down and the ADP concentration went up.

Possible interpretations of IV

(a) ATP is responsible for muscle contraction.

(b) The breakdown of ATP provides the energy for rebuilding creatine phosphate from creatine and inorganic phosphate.

(c) The breakdown of creatine phosphate is not an essential step in providing the energy for muscle contraction.

(d) No unequivocal interpretation is possible.

Experimental evidence V

1 By 1947, it was known that glycogen was converted into glucose, which was then broken down in a series of reactions to pyruvic acid and this, in turn, gave rise to lactic acid in anaerobic conditions. The action of iodoacetic acid referred to in III (a) was to block one of these reaction steps.

2 One of these intermediate steps was an oxidative one which was coupled with the synthesis of ATP from ADP.

3 Szent-Györgyi in 1948 showed that placing ATP on appropriately prepared muscle fibres would cause them to contract.

4 Frog muscle contains relatively little ATP. The level of creatine phosphate is several times higher.

5 During 'normal' contractions, the ATP level remains unchanged except in extreme fatigue.

On the basis of all the evidence presented so far, draw a flow chart showing the intermediate steps from glycogen to lactic acid. Indicate the point at which ATP is formed and show a connection between ATP and contracting muscle. Indicate on the chart the role you think is played by (a) creatine phosphate, (b) oxygen, and point out the stages at which iodoacetic acid acts as an inhibitor.

When you have completed this exercise, consider your conclusions in the light of the following comments.

I Interpretation (c) fits the evidence of items 2, 3, and 4. For the other interpretations, note:

(a) This is contrary to the evidence of item I.1.

(b) This is contrary to the evidence of I.4. It is *not* stated there that *no* lactic acid is formed, only *less*.

(d) This would fit the facts, but no evidence is offered in I concerning the oxidation of lactic acid.

(e) This was once thought to be the case, but on the basis of the observations presented, looks rather unlikely. If lactic acid *causes* contraction, why should the muscle become 'fatigued' when the level of lactic acid goes up to a certain level? It might be that a low concentration stimulates contraction but a high concentration produces fatigue. Other experiments, not mentioned here, have shown that lactic acid accumulates after the contraction. This would provide additional evidence for ruling it out as a *cause* of contraction.

II Whilst all three interpretations (a), (b) and (c) are supported by the evidence, in no case is there adequate data to be conclusive about them. Although (c) might be seen as the best 'hunch' or working hypothesis, (d) remains the most accurate statement, strictly speaking. Most working scientists of the time would have opted for the non-rigorously established (c). If you are uncertain about this consider the following.

(a) The chemical equivalence does not establish that the lactic acid is a product of glycogen. A glycogen molecule could *act on* another substance which then produces one or more molecules of lactic acid. Knowledge of the chemical composition of glycogen and lactic acid would indicate whether the conversion of glycogen to lactic acid is *likely*, and the sort of isotope labelling experiment discussed in section 15.5 would confirm it.

(b) On the evidence presented here, such a conclusion is not really acceptable. The disappearance of glycogen during contraction does not prove that it is chemically degraded, though a knowledge of its chemistry makes this a reasonable guess. It is plausible that glycogen could release energy if broken down.

(c) The clearly established proportionality between glycogen disappearing, lactic acid accumulating, and muscular pull makes this an acceptable interpretation, but it is still unsatisfactorily vague. Nevertheless, it will serve for the time being, and in fact by 1927, in the light of this and other evidence, it was generally accepted that the breakdown of glycogen to lactic acid provided the energy for muscle contraction. In this sense it is a preferable interpretation to (d).

III (a) The only doubt as to this being the case might be that glycogen is not necessarily the only source of lactic acid but, if, as stated in III.2, no lactic acid is formed, it follows that glycogen cannot be broken down to it.

(b) It depends on what one means by 'responsible'. Creatine phosphate seems to be connected with contraction but (i) it could be broken down as a result, rather than as a cause, of muscle contraction, (ii) it might be responsible, but in a very indirect way.

(c) The evidence of III.3 indicates that creatine phosphate is resynthesized in normal muscle at rest. It looks as though the blockage of glycogen → lactic acid (admittedly not fully established by our evidence) is associated with the fact that creatine phosphate is not resynthesized. This assumes, however, that the iodoacetic acid has not interfered with other chemical changes which might play a part in the creatine phosphate cycle. Although interpretation III (b) is not entirely acceptable for these reasons, it would form a useful working hypothesis and a sound basis for further investigations. (Note: Lundsgaard's experiment was important in disposing of the idea that lactic acid was in some way responsible for muscle contraction.)

IV (a) The reservation expressed in comment III (b) above must be repeated. Evidence IV.3, however, indicates that when both the reactions glycogen → lactic acid and creatine phosphate → creatine + phosphate are stopped by an inhibitor, muscle can still contract and ATP is broken down. Possibly, then, ATP is a little closer to the immediate mechanism of contraction than either lactic acid or creatine phosphate.

(b) If the creatine phosphate breakdown is blocked by an inhibitor the number of contractions is greatly reduced. Thus it could be that normally CP recharges ATP from ADP and inorganic phosphate. Evidence IV (b) indicates that such a transfer can take place and that, at least in experimental conditions, CP would only breakdown if ADP were present.

(c) Since, in the presence of the inhibitor, the level of CP remains the same while the muscle still manages to contract, CP is not an essential step (in the experimental situation anyway); but the number of contractions is greatly reduced, making it appear that, normally, CP has an important part to play in muscle contraction.

V On the basis of the evidence presented in V and the preceding results, you could construct the flow-chart (Fig. 8) for anaerobic conditions. Creatine phosphate is here shown as functioning as a 'deposit' of an 'energy-rich' molecule which can be converted into ATP as in IV.2, and accounts for the maintenance of ATP levels during contraction mentioned in V.4 and 5. (See also 15.4.)

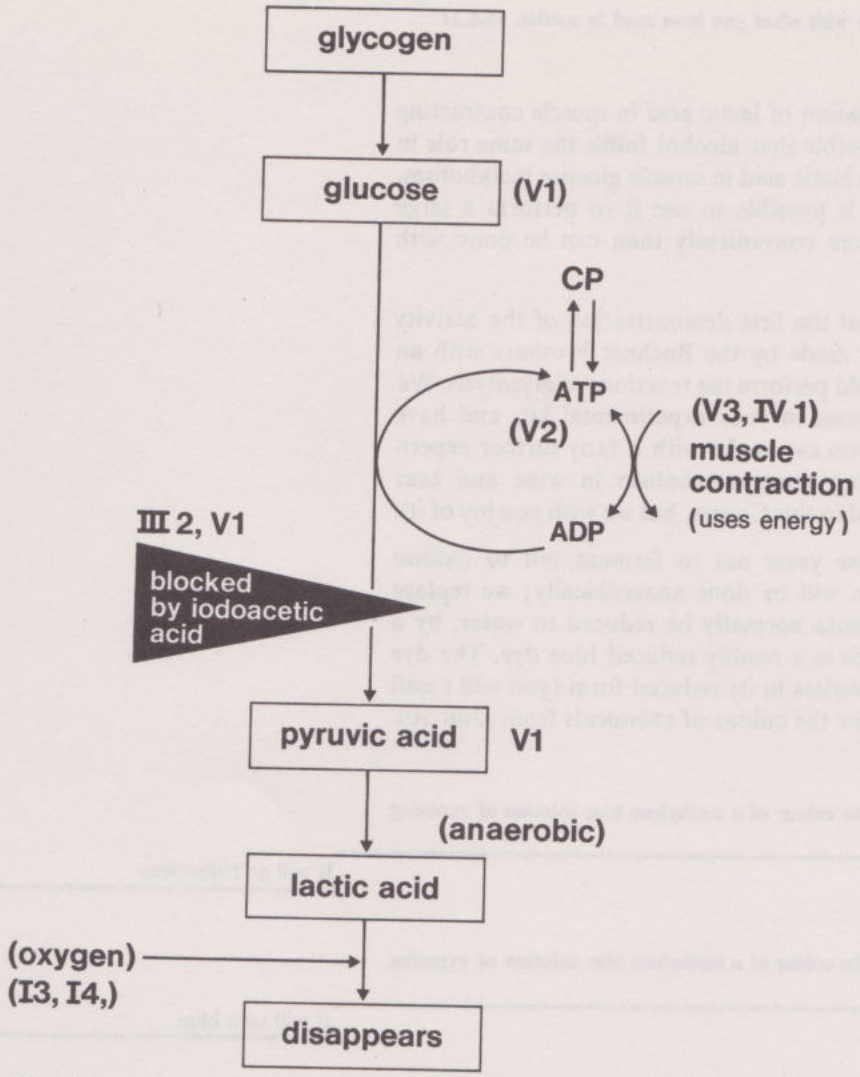


Figure 8

15.6.2 An experiment in glucose metabolism

glucose metabolism

We said in section 15.6 that much of the evidence concerning the pathway of glucose metabolism had been derived from an examination of the reactions occurring in muscle and yeast. Some of the evidence that led to our conclusion concerning muscle glycolysis was presented in section 15.6.1. That yeast breaks down glucose has been known since antiquity, for alcohol, one of the products of the activity of this micro-organism, has long been of interest both to scientists and non-scientists for its interesting psychopharmacological effect when taken orally. Anyone who has ever made wine or beer at home will know that one of the keys to a successful fermentation is that the yeast is allowed to act on a warm, sugary solution, anaerobically (in the absence of air). Under these circumstances, alcohol begins to accumulate in the brew, and its final concentration may be as high as 12–15 per cent before fermentation ceases. Alcohol, then, is one product of the anaerobic glycolysis of glucose by yeast. A second will also be familiar to wine or beer brewers.

Why does the fermentation mixture bubble?

During the reaction sequence carbon dioxide is released; it is the CO_2 coming off the wine or beer brew which makes it sparkle, or fizz.

If the yeast is allowed to react with the glucose in the presence of oxygen, however, alcohol does not accumulate in the same way as it does anaerobically.

Does this suggest an analogy with what you have read in section 15.6.1?

The analogy is with the accumulation of lactic acid in muscle contracting anaerobically. It would seem possible that alcohol fulfils the same role in yeast glucose metabolism as does lactic acid in muscle glucose metabolism. Yeast is convenient, because it is possible to use it to perform a large number of metabolic studies more conveniently than can be done with animal tissues.

You will recall from Unit 14 that the first demonstration of the activity of enzymes outside the cell was made by the Buchner brothers with an extract from yeast cells which could perform the reactions of glycolysis. We have provided you with dried yeast in your experimental kit, and have suggested one such experiment you can make with it (any further experience you might gain in handling yeast metabolism in wine and beer making must count as extra-mural to this Course, but we wish you joy of it).

In this experiment, you will use yeast not to ferment but to oxidize glucose. However, the oxidation will be done anaerobically; we replace the oxygen of the air, which would normally be reduced to water, by a substance, methylene blue, which is a readily reduced blue dye. The dye is blue in its oxidized form, colourless in its reduced form (you will recall some of the molecular reasons for the colour of chemicals from Unit 10).

What will be the effect on the colour of a methylene blue solution of exposing it to a reducing agent?

It will go colourless.

What will be the effect on the colour of a methylene blue solution of exposing it to an oxidizing agent?

It will turn blue.

Predict the effect of adding glucose to a methylene blue solution. (Note: consider whether glucose is a reducing or an oxidizing agent, or neither.) Predict the effect of adding yeast alone to a methylene blue solution.

In the experiment which follows, you will use the *rate of decolorization* (the time taken for the methylene blue to turn from blue to colourless) as a measure of the speed of the reactions. The experiment is thus formally similar to those on salivary amylase of section 15.3.1. The difference is that in the case of the amylase you were examining the factors affecting a single enzyme reaction. In these experiments you are examining a sequence of reactions, catalysed by a set of enzymes acting in series upon the glucose molecule and its products.

Predict the effect of adding a mixture of glucose and yeast to a methylene blue solution.

On the basis of what you have so far read in this Unit, you are in a position to make two other predictions. What would you predict the effect to be of adding to the glucose/yeast/methylene blue mixture a solution of:

(a) iodoacetic acid?

(b) magnesium sulphate?

If you are not sure, refer back to the experimental evidence presented in section 15.3.1 and the dicussion of factors affecting enzyme reaction rates in *The Chemistry of Life*, pp. 88–100 and section 15.3.

Having made your predictions, now test them by experiment. (Note: because of the time involved, read through the whole experiment first, and plan your tactics appropriately.)

Apparatus required

1 Conical flasks
2 Beakers
3 Plastic syringes
4 Test-tubes and corks
5 Stopwatch

Chemicals required

1 Dissolve 2.5 g of glucose in 50 cm^3 of distilled water in a conical flask.

2 Dissolve 0.18 g of iodoacetic acid in 50 cm^3 of distilled water in a conical flask (CARE!—wash your hands after doing this).

3 Dissolve 0.24 g of magnesium sulphate in 50 cm^3 of distilled water in a conical flask.

4 Dissolve 0.1 g of methylene blue in 100 cm^3 of distilled water in a conical flask.

5 'Freshen up' your dried yeast preparation by adding 10 g to a solution containing 1 g of glucose in 50 cm^3 of distilled water. Leave for an hour or more before using.

Note: the weights in this experiment are not critical and you need only be approximate.

Have your stopwatch ready, and prepare 6 test-tubes as follows (figures are in cm^3).

Table 6

Tube	1	2	3	4	5	6
H_2O	13	10	8	5	—	—
Glucose	—	—	5	5	5	5
Iodoacetic acid	—	—	—	—	5	—
Magnesium sulphate	—	—	—	—	—	5
Yeast	—	3	—	3	3	3

Now add 1 drop of methylene blue to each tube, cork, mix by briefly shaking, and start your stopwatch. Which tubes go colourless, and how long do they take to do so? Note that different yeast samples may vary in the ability to carry out these reactions depending on a variety of factors, including the effectiveness of your 'freshening-up' procedure. In our experiments at the Open University, some tubes went colourless in about 20–30 minutes. Others did not go colourless for as long as we left them (up to 5 hours). Uncork the tubes that have gone colourless and leave them undisturbed and exposed to the air for a few hours. Do you observe any change? If so, how do you account for it?

We do not expect you will have time to make more detailed observations on this experimental situation. If you do, however, you might like to consider the following additional questions.

What is the effect of replacing the glucose used in this experiment with the starch solution you prepared for the experiments of section 15.3.1?

What is the effect of varying the incubation temperature on the reaction rates?

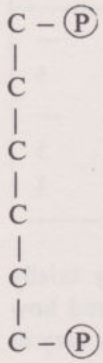
How did the results of these experiments match your predictions?

15.6.3 The glycolytic sequence

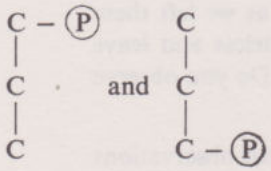
The experiments you have read about and made in sections 15.6.1 and 15.6.2 told the researchers of the 1920s and 1930s something of the overall reaction sequences involved in glucose metabolism. It required a combination of these, and the purification of the individual enzymes and coenzymes of the glycolytic and glucose oxidation pathways, to make up a picture of the full sequence of reactions. Bear this, and the flow diagram you constructed in section 15.6.1 in mind, in what follows. Note also that the first part of this reaction sequence, from glucose to pyruvic acid, is identical not only in muscle and yeast, but in *all* animal and plant tissues that have so far been examined. It seems to be a universal mechanism for glucose metabolism.

We shall not give the full reaction sequence involved in glycolysis. It is given in *The Chemistry of Life*, pp. 122–128; glance at this, but *do not* attempt to remember the details of the sequence or the names of individual enzymes. Do remember, however, that *each* of the reactions described is catalysed by a specific enzyme. For those of you going on to study Science next year, though, this is black-page material.

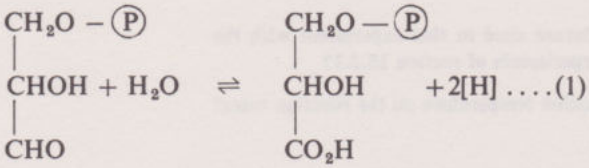
In summary, the reactions proceed as follows. (Note that each is catalysed by a particular enzyme, which has been purified and studied, but which we will 'take for granted' in all the reactions described in the rest of this Unit). To start glucose metabolism going, the glucose molecule must be activated. This is achieved, like many such cellular reactions, by *phosphorylating* the glucose molecule at the 1 and 6 positions. The phosphate groups are derived from two molecules of ATP, which are converted to two of ADP. Thus, in a reaction sequence which will eventually result in ATP *synthesis* by the cell, the initial steps actually *use up* ATP. The sugar, which can now be represented as follows (we draw the molecule as a *chain* rather than as a ring for the sake of clarity):



This molecule is then split in half to yield two 3-carbon molecules, each containing one phosphate group.



These fragments are oxidized (by removal of hydrogen, Unit 8) to a 3-carbon acid:



3-phosphoglyceraldehyde *3-phosphoglyceric acid*

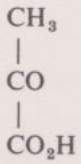
This reaction, which is complex, is coupled (by way of the type of phosphorylated intermediate discussed in section 15.4) with the conversion of 1 molecule of ADP and 1 of phosphate to 1 of ATP. The reaction is an oxidative one, and was found to require a low-molecular weight coenzyme (see section 15.3), a substance we shall refer to as NAD, short for nicotinamide adenine dinucleotide. Its formula, which you should not attempt to remember, is to be found on p. 113 of *The Chemistry of Life*. NAD can be reversibly oxidized and reduced (Unit 9),

$$NAD + 2\,H \rightleftharpoons NADH_2 \quad \ldots\ldots\ldots (2)^*$$

and the oxidation of the 3-phosphoglyceraldehyde, shown in equation 1, is coupled to the reduction of NAD to $NADH_2$. We shall consider the fate of $NADH_2$ shortly.

A series of four further reactions now converts the phosphoglyceric acid to *pyruvic acid*

pyruvic acid



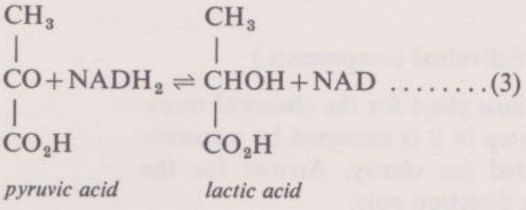
In the course of this reaction sequence, the phosphate attached to the phosphoglyceric acid is transferred to ADP, synthesizing a further molecule of ATP.

What is the net yield of ATP from 1 molecule of glucose so far?

Two.

Two ATPs are required to phosphorylate the glucose to begin with: each glucose molecule yields 2 molecules of pyruvic acid.

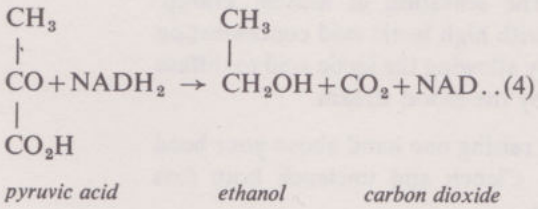
In the absence of oxygen, the reaction sequence ends here. During it, a molecule of NAD has been reduced to $NADH_2$. In muscle and other animal and plant tissues, the NAD is now regenerated through a reaction in which pyruvic acid is reduced to lactic acid.



pyruvic acid *lactic acid*

Lactic acid is thus the *end product* of anaerobic glycolysis in animals, as you saw from the experiment with muscle in 15.6.1. You will note that the coenzyme for reaction (3) is NAD once again, and during the reaction $NADH_2$ is reoxidized to NAD.

In yeast, an alternative fate awaits the pyruvic acid. It is broken down by way of a further series of reactions into a molecule of carbon dioxide and one of ethanol, that is, alcohol.



pyruvic acid *ethanol* *carbon dioxide*

This is the fermentation reaction which distinguishes glycolysis in yeast from that in animals. Other micro-organisms also perform comparable

* *This equation and our subsequent use of '$NADH_2$' is something of a simplification, as you will see if you follow the black-page material of* 'The Chemistry of Life'.

fermentation reactions, resulting in a variety of end-products of glucose breakdown. Some micro-organisms, though, will generate lactic acid. Indeed, its name indicates its origin in milk (Latin: *Lac*, milk) where it accumulates during 'souring' or the fermentation which converts milk to yoghurt.

The reaction sequence is shown schematically in Figure 9, and in more detail in Figure 10.

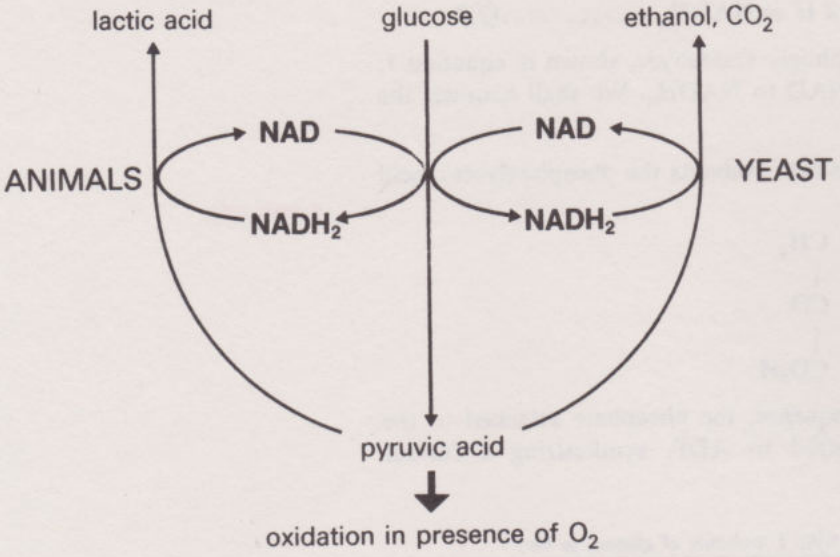


Figure 9 Glycolysis in animals and yeast.

Study Comment

Figure 9 represents the key points which you should be able to recall following your reading of sections 15.6.1, 2 and 3. Figure 10 shows the full sequence.

(Do *not* attempt to recall the names of individual components.)

This diagram is included to serve as a flow chart for the chemical transformations that occur. Note that each step in it is catalysed by a specific enzyme, whose name has been omitted for clarity. Arrows for the coenzymes are shown in the breakdown direction only.

What happens to the lactic acid? The animal cell cannot afford to let large concentrations of lactic acid build up within it. This is both because several of the glycolytic reactions are reversible (and if too much of the end products began to accumulate, they would begin to run the wrong way, due to a concentration effect, Unit 12) and because the presence of an acid in large concentrations would result in large changes in pH that would be damaging to the cell. Most of you will have experienced, in mild form at least, this damaging effect. The sensation of muscle 'cramp' following violent exercise is associated with high lactic acid concentration in the muscle. This problem is avoided by allowing the lactic acid to diffuse out of the cell and to be washed away by the blood stream.

You can study this effect very simply by raising one hand above your head and holding the other hanging down. Clench and unclench both fists rapidly over a period of several minutes.

Which one tires first?

You should find that it is the one above your head.

What form does the 'tiring' take?

For most people, a muscular ache, a 'cramp'.

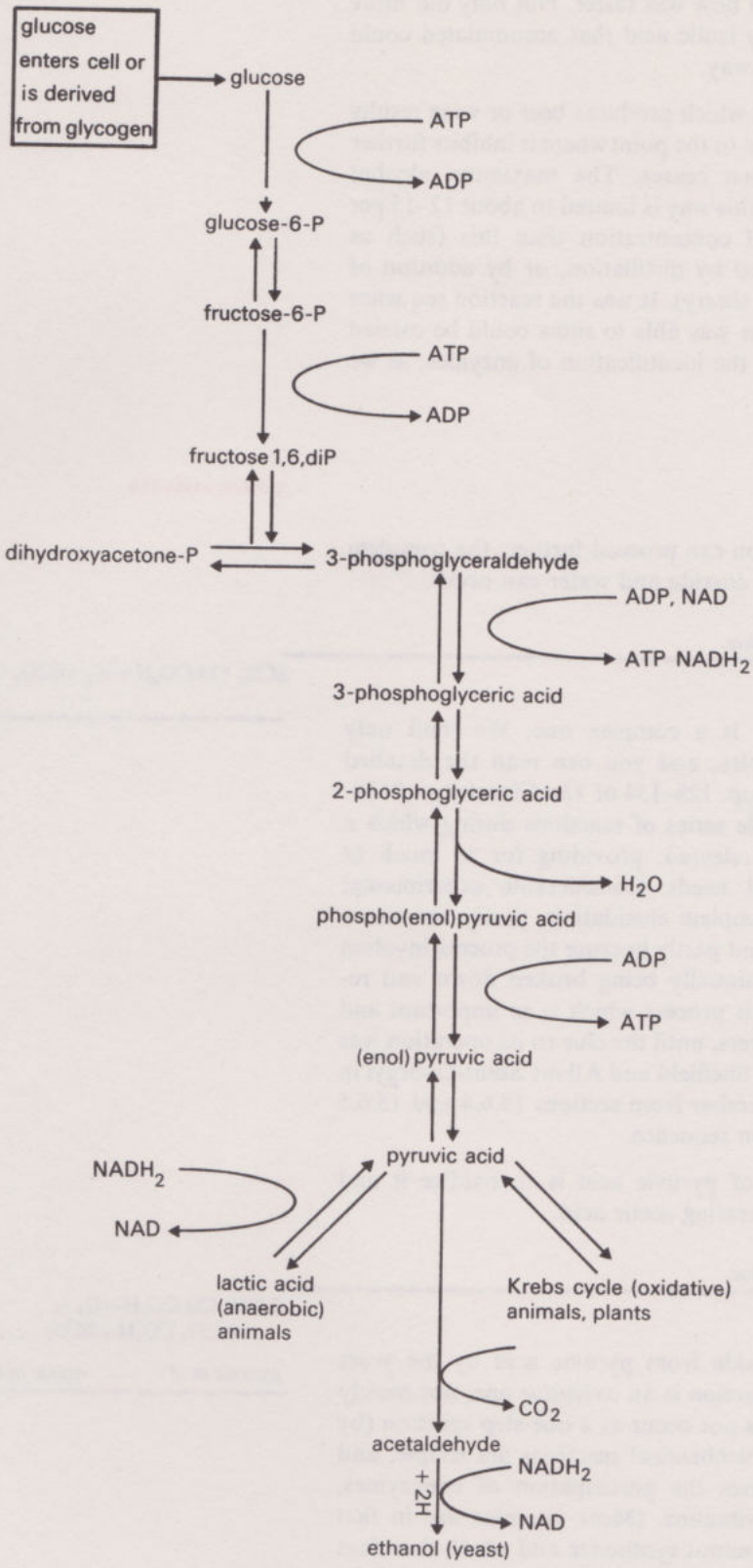


Figure 10 *The glycolysis sequence.*

Can you suggest any explanation for the difference between the behaviour of the two arms? Might it be due to a difference between the two hands, irrespective of the arm position? How could you check?

The hand held above your head has a diminished blood flow compared with that which hangs below your wrist; less blood means less oxygen arriving at the muscles of the hand, and hence a greater generation of lactic acid. Because of the blood flow, the lactic acid tends to accumulate, and it is this accumulation which is associated with the 'cramp' sensation.

In the arm which hangs down, blood flow was faster. Not only did more oxygen arrive at the muscle, but any lactic acid that accumulated could diffuse out of the cell and be swept away.

In the case of yeast, the fermentation which produces beer or wine results in an increase in alcohol concentration to the point where it inhibits further enzyme reactions. Fermentation then ceases. The maximum alcohol concentration that can be built up in this way is limited to about 12–15 per cent. Drinks with a higher alcohol concentration than this (such as whisky and gin) have to be prepared by distillation, or by addition of distilled liquor ('fortified' wines, like sherry). It was the reaction sequence of fermentation in yeast that Buchner was able to show could be carried out in a cell-free system, and led to the identification of enzymes, as we discussed in Unit 14.

15.6.4 Glucose oxidation

glucose oxidation

In the presence of oxygen, the reaction can proceed further: the complete oxidation of pyruvic acid to carbon dioxide and water can occur.

Write the equation for this oxidation.

$2CH_3\,CO.CO_2H + 5O_2 \rightarrow 6CO_2 + 4H_2O$ .....(5)

Once again, the reaction sequence is a complex one. We shall only summarize here the general principles, and you can read the detailed account (as black-page material) on pp. 128–134 of *The Chemistry of Life*. The full mechanism involves a whole series of reactions during which a considerable amount of energy is released, providing for as much as three-quarters of the cell's normal needs. Considerable experimental difficulties lay in the way of its complete elucidation, partly because a number of co-factors are involved, and partly because the process involves a cycle of reactants which are continually being broken down and re-formed. It is the cyclic nature of this process which is so important and which for so long baffled experimenters, until the clue to its operation was provided in 1937 by Hans Krebs* at Sheffield and Albert Szent-Györgyi in Budapest. You are required to remember from sections 15.6.4 and 15.6.5 only the cyclic nature of this reaction sequence.

The first stage in the breakdown of pyruvic acid is to oxidize it and remove carbon dioxide from it, generating acetic acid.

Write the equation for this reaction.

$2\,CH_3\,CO\,CO_2H + O_2 \rightarrow 2\,CH_3\,CO_2H + 2CO_2$ .....(6)

pyruvic acid *acetic acid*

Unlike the removal of carbon dioxide from pyruvic acid by the yeast enzymes (equation 4) the present reaction is an *oxidative* one, not merely a removal of carbon dioxide. It does not occur as a one-step reaction (by now you must be feeling that few biochemical reactions are simple, and you are probably right!). It requires the participation of coenzymes, including one of the B group of vitamins. (Most vitamins are in fact enzyme co-factors which the body cannot synthesize and which therefore must be obtained from an external source, that is, as components of the organism's diet.)

We have written the reaction as a straightforward oxidation, but in fact, like the oxidation of 3-phosphoglyceraldehyde (equation 1); it uses the coenzyme NAD as a recipient of hydrogen atoms. Other coenzymes are also involved, and the details are discussed (black pages) in *The Chemistry of Life*, pp. 128–130.

* *Krebs talks about his role in this discovery in the radio programme of Unit* 15.

15.6.5 The Krebs cycle

the Krebs cycle

By oxidizing pyruvic to acetic acid, we have removed one CO_2 molecule and produced one molecule of reduced $NADH_2$. It still remains to oxidize the remaining two carbon atoms of the acetic acid to carbon dioxide. It is here that we enter into a cycle of reactions that is associated with the name of Hans Krebs.

The essential feature of the cycle is that the 2-carbon fragment of acetic acid is made to combine with a 4-carbon acid, to yield a 6-carbon acid. The 6-carbon acid is broken down in steps to give, first, a 5-carbon acid, and then a 4-carbon acid. Two molecules of CO_2 are given off in the process. The 4-carbon acid is then converted into the form necessary to recombine with acetic acid. At various stages in the process, oxidation steps result in the production of $NADH_2$ from NAD, and ATP from ADP. The useful thing about this merry-go-round, as far as the cell is concerned, is that it provides that critical feature of all desirable energy-releasing systems, a way for the acetic acid molecule to be picked carefully to pieces so as to enable its oxidation to be coupled with the synthesis of ATP from the ADP's inorganic phosphate. The outline of the scheme is shown in Figure 11.

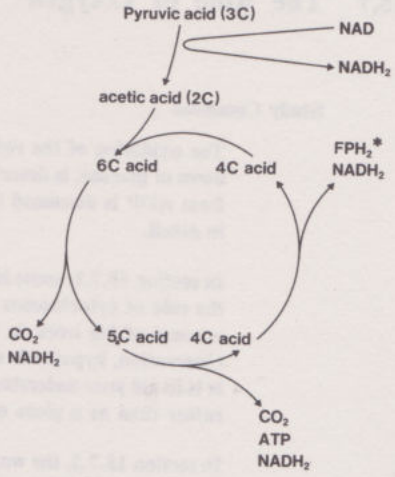


Figure 11 *The Krebs cycle.*

You can read the full details of the Krebs cycle as black-page material on pp. 131–134 of *The Chemistry of Life.*

By means of the cycle, then, acetic acid from pyruvic acid can be completely oxidized in a stepwise reaction, which also produces a number of molecules of the reduced coenzyme $NADH_2$. These reactions, however, demand a continuous supply of NAD, as it is being continuously removed as $NADH_2$. We have seen that, under anaerobic conditions, some NAD can be regenerated by reducing pyruvic acid to lactic acid. But this mechanism cannot cope with the quantities of $NADH_2$ generated by the Krebs cycle. The $NADH_2$ must be reoxidized by molecular oxygen. The sequence of oxidation steps involved in this process is the major energy-yielding system of the cell. It is discussed in the next section.

Before leaving the Krebs cycle, we should emphasize its central role in cell energetics. The bulk of the cell's ATP is generated through oxidation of glucose; but not only glucose catabolism goes by way of the cycle. The breakdown of fats and amino acids also results in the production of substances which are intermediate members of the Krebs cycle, and can hence be oxidized through it. We do not propose to enter into a discussion of this interlaced web of reactions here, but an indication of the central role of the cycle will be gained if you look at Figure 7 again, and observe the number of inputs into the cycle. It is a reasonably accurate generalization to suggest that, for most of the oxidative catabolic reactions of the cell, the final break-up of the carbon skeleton of the molecule occurs whilst whirling round the Krebs cycle merry-go-round (once again, you can read about some of the details of these reactions in pp. 137–149 of *The Chemistry of Life*, as black-page material). You will see in Unit 16 how many of the major synthetic reactions of the cell also involve intermediates of the Krebs cycle.

* *FPH_2 is another reduced co-factor analogous to $NADH_2$.*

15.7 The Role of Oxygen

Study Comment

The oxidation of the reduced coenzyme $NADH_2$, generated during the breakdown of glucose, is described. The coupling of this oxidation with ATP synthesis from ADP is discussed (section 15.7), and should be understood but not learnt in detail.

In section 15.7.1, some historical background to the discovery and elucidation of the role of cytochromes in cells is given by means of an extract from Keilin's account of his work in this field. The roles of, and the relationship between, observation, hypothesis and experiment are clearly brought out in this account. It is to aid your understanding of how scientists work that the extract is included, rather than as a piece of material to be memorized.

In section 15.7.2, the way in which the breakdown of molecules is linked with the production of ATP is outlined in more detail. It is important to understand the implications of the process, rather than to learn the details.

The cellular sites at which the reactions discussed in this section of the Unit take place, have been identified. The function of mitochondria, which you studied in Unit 14, is explained and related to their structure—so reinforcing the general theme of Unit 14. It is important to understand this general concept.

You probably know, from general knowledge, how oxygen is transported to the cell. How?

In man, oxygen is taken up from the air by way of the lungs, and enters the blood stream to form a loose combination with the red, iron-containing blood pigment, haemoglobin†. When the circulating blood reaches the body tissues where the amount of oxygen is low, the haemoglobin-oxygen complex dissociates, releasing oxygen into solution, whence it can diffuse through capillary walls and into cells. In return, carbon dioxide produced by the cells passes into the blood stream and is swept away to the lungs, where it is released into the air. Thus the cell is kept constantly supplied with enough oxygen to help generate the energy it needs. The processes of obtaining oxygen from the surrounding atmosphere and utilizing it within the organism are collectively known as *respiration* (Unit 18). Here we are concerned with the reactions involving oxygen that occur within the cells, and are sometimes collectively referred to as *cell respiration*.

cell respiration

We have already referred to oxidation earlier in this Unit, and the general mechanism and significance of oxidative reactions were discussed in Unit 8. Of the types of oxidation reaction available to the cell, direct oxidation by molecular oxygen of the form:

$$A + O_2 \rightarrow AO_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

is rarely carried out by the cell: such reactions in general only occur in the cell at the end of a sequence of enzyme-catalysed transfers. There are, however, enzymes called oxidases,† which catalyse the direct oxidation of their substrates by atmospheric oxygen. They are found in most cells, and are especially common in plants. The effects of one of them, polyphenol oxidase, are familiar to those who peel their apples before eating them. Apples contain traces of a compound called catechol which is oxidized, under the influence of polyphenol oxidase, to a complex, dark brown substance. When an apple is cut, its surface is exposed to the air and

polyphenol oxidase can begin to work, turning the surface of the apple gradually brown.

In general the cell oxidations we deal with are of two types. The first is of the form

$$AH_2 + B \rightarrow A + BH_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots (8)$$

where A is oxidized at the expense of another substance, B, which is reduced. The second involves an electron transfer, e.g., the oxidation of one ionic form of iron (Fe II) to another (Fe III)*

$$Fe^{++} \rightarrow Fe^{+++} + e \quad \ldots\ldots\ldots\ldots\ldots\ldots (9)$$

Recall a reaction of the form of equation 8 from this Unit.

The reactions involving the reduction of NAD to $NADH_2$ during glycolysis and glucose oxidation are of this form.

Enzymes which carry out reactions of the type of equation 8 are called *dehydrogenases*,† and during the reactions of glycolysis and the Krebs cycle a series of these dehydrogenases act upon the intermediate substances, dehydrogenating (oxidizing) them, at the expense of NAD. $NADH_2$ must subsequently be reoxidized, and it is during this sequence of reactions that the major portion of cellular ATP generation occurs.

dehydrogenase

The overall reaction

$$NADH_2 + \tfrac{1}{2}O_2 \rightarrow NAD + H_2O \quad \ldots\ldots\ldots (10)$$

is one in which a large amount of energy is released. It occurs in a series of enzyme-catalysed steps which are directly coupled to ATP production**. The salient points which we need to bring in here are that the sequence of intermediates is arranged so that each time the hydrogen atoms are passed from one to another, a little energy is released and can be tapped off as ATP. The sequence is known as the *hydrogen transport chain* (sometimes called the *electron transport chain*). At the very end of the chain of oxidations, oxygen itself must of course be involved as a reactant, or there would be no way for the last hydrogen carrier†, Z, say, to get rid of its hydrogen once it had been reduced. So the last carrier of all is oxidized by oxygen, the reaction being catalysed by an oxidase, thus:

hydrogen transport chain

$$ZH_2 + \tfrac{1}{2}O_2 \rightarrow Z + H_2O \quad \ldots\ldots\ldots\ldots (11)$$

We start, in this process, then, with a substrate, AH_2, whose oxidation would release a substantial quantity of energy, and pass its hydrogen down a chain of carriers, each time releasing a little energy, until at the very end the last carrier transfers it to oxygen and thus disposes of it as a molecule of water. The process can be compared with one of the water-wheels that used to be used for grinding corn. Water pours on to the top bucket of the water-wheel at high potential energy, as it has several feet to fall. The first bucket breaks its fall. Some of the potential energy of the water is converted into kinetic energy of the bucket, which begins to move, carrying the wheel around. The water spills into the second bucket, yielding more energy and hence more movement, then into the third, the fourth, and finally into the stream at the bottom; during its fall down the

* *These reactions are analogous to other electron-removal oxidations you met in Unit* 8, *although the specific case of the ions of iron there was not considered.*

** *The details of the sequence have not been fully worked out, and are more complex than need concern us here. They are given on pp.* 113–118 *of* 'The Chemistry of Life', *which may be read as* black-page *material.*

series of buckets, its potential energy has been tapped off at several points and used to spin the wheel. As with the water, so the hydrogen in its fall towards oxygen is checked at several points and its available energy tapped off.

Between NAD and molecular oxygen there are at least five intermediate carriers that are alternately oxidized and reduced in the sequence shown in equation 12, where we represent the carriers by the letters V-Z

$$\begin{array}{ccccccccccc} NADH_2 & & V & & WH_2 & & X & & YH_2 & & Z & & H_2O \\ & \searrow & & \nearrow & & \searrow & & \nearrow & & \searrow & & \nearrow & \\ NAD & & VH_2 & & W & & XH_2 & & Y & & ZH_2 & & \tfrac{1}{2}O_2 \end{array} \quad \ldots(12)$$

In actual fact, the second portion of this sequence of oxido-reduction occurs as electron transport rather than hydrogen transport. Carriers X, Y and Z are substances known generally as *cytochromes*, iron-containing coloured substances closely related in chemical structure to haemoglobin. During the sequence of oxidation and reduction, the iron ion in each cytochrome is alternately in its Fe (III) (oxidized) and Fe (II) (reduced) form; only with the last cytochrome in the series, carrier Z of equation 12 (actually known as cytochrome a), does a reaction occur in which the reoxidation of the Fe (II) ion to the Fe (III) ion involves oxygen, which is simultaneously reduced to water (equation 13).

cytochrome

$$\text{(cyt) } Fe^{++} + H^+ + \tfrac{1}{2}O_2 \rightleftharpoons \text{(cyt) } Fe^{+++} + H_2O \quad \ldots(13)$$

This is the terminal step of cell oxidation; it is perhaps of interest that the well-known poison, potassium cyanide, acts by inhibiting the terminal cytochrome oxidation.

The discovery of the mechanism of oxidation through the hydrogen transport chain of the cell makes one of the more fascinating chapters in the history of biochemistry. The cytochromes were discovered in the late nineteenth century by a Scottish country doctor, an amateur scientist, but his work was viewed sceptically by one of the leading organic and physiological chemists of the time, the German Hoppe-Seyler, who claimed that the observation was simply due to contamination of the samples with the blood pigment haemoglobin. Hoppe-Seyler's prestige was so great in the scientific community that the results were disregarded, and it was not until the 1920s that the cytochromes were independently rediscovered by David Keilin, at Cambridge, who examined their absorption spectra, using a spectroscope very similar to the one you experimented with in Units 6 and 7. Keilin directed this spectroscope to the muscles of a fly, which certainly did not contain haemoglobin, and found absorption lines of the sort shown in Figure 12. Keilin repeated his observation with other organisms such as yeast, and you may care to repeat his experiment (you will see how in a moment) using your own spectroscope.

15.7.1 An experiment with cytochromes

Keilin's description of his own observations is so clear that it is worth quoting in full. Note that Keilin is very concerned, in the description, to prove that the cytochromes are quite distinct from the blood pigment haemoglobin. Note how, having set up the hypothesis that they *are* distinct, he then attempts to *disprove* it by as carefully controlled a set of experiments as possible:

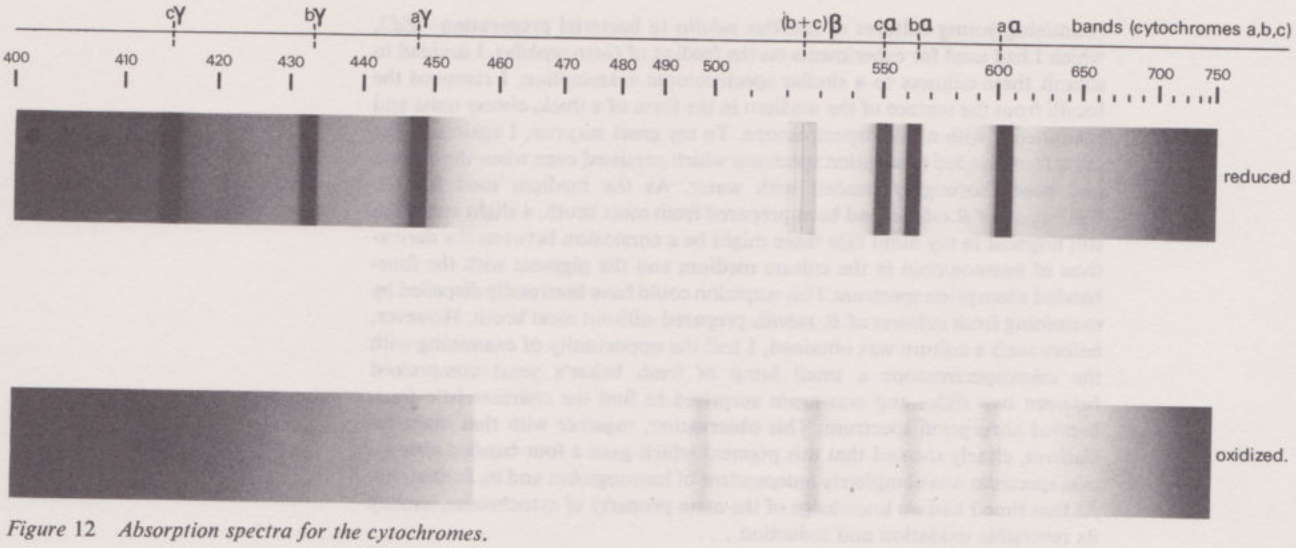


Figure 12 Absorption spectra for the cytochromes.

The discovery of cytochrome in adult *Gasterophilus* and other insects and in cells of bacteria and yeast.

The study of the . . . fate of haemoglobin in the pupa and adult *Gasterophilus* (a type of fly whose larva parasitises the horse and which, therefore, might be contaminated with horse haemoglobin—*Ed.*), was more successful and yielded results which were much more important than I could have anticipated. In the first batch of *Gasterophilus* pupae, obtained early in the summer of 1923, I detected a gradual disappearance of larval haemoglobin, especially during the later stages of metamorphosis. From the pupae a number of adult flies emerged and these were kept under observation as long as possible, especially as it was my first experience of breeding these flies under laboratory conditions. Spectroscopic examination of the tissues of adult *Gasterophilus* which had died in captivity revealed the presence in the thoracic muscles of a pigment with four distinct absorption bands. At first I thought that this pigment had originated from the larval haemoglobin and had been further modified by keeping the adult insects under artificial conditions. However, numerous spectroscopic experiments were carried out on this pigment and on the derivatives obtained from it when the muscle tissues were treated with acids, alkalis and organic solvents, but I could obtain no clear indication as to its relationship to the larval haemoglobin. Having exhausted the material available in 1923, further study of this pigment was put off for another year.

Early in the summer of 1924 I obtained a large number of *Gasterophilus* pupae and from these there emerged several perfectly formed adult flies. Spectroscopic examination of the thoracic muscles of fresh specimens of these flies revealed the same four-banded absorption spectrum that I had noticed the previous summer. This time there was no doubt that the pigment formed an integral part of the fresh muscle tissue of this fly. In order, however, to determine the relationship between this pigment and larval haemoglobin, I examined spectroscopically the thoracic muscles of other insects, the larvae of which are devoid of haemoglobin. The first insect selected for this purpose was the adult blowfly (*Calliphora erythrocephala*). The fact that the thoracic muscles of this fly also exhibited the same four-banded spectrum, although it greatly weakened the case for an interrelationship between the same pigment in adult *Gasterophilus* and the haemoglobin of its larva, did not, however, completely dismiss such a possibility because the blowfly larvae had been fed on meat rich in haemoglobin. As a more reliable control I then selected the adult wax-moth (*Galleria mellonella*), which had been bred in the laboratory from a rich culture of its caterpillars fed on old honey-combs. The thoracic muscles of this insect, which had not been in contact with haemoglobin at any stage in its development, nevertheless displayed the same four-banded absorption spectrum as that shown by the thoracic muscles of adult *Gasterophilus* and *Calliphora*. While I was trying to find in the food of *Galleria* any elements which might possibly be connected with the pigment in the muscles, I dissected a number of *Galleria* caterpillars, but careful examination of their intestinal contents revealed no characteristic pigment. They contained only some light-brown granules, particles of wax, pollen, yeast and bacteria. However, as I had at that time several large dishes

containing young cultures of *Bacillus subtilis* (a bacterial preparation—*Ed.*), which I had used for experiments on the feeding of *Gasterophilus*, I decided to submit these cultures to a similar spectroscopic examination. I removed the bacilli from the surface of the medium in the form of a thick, cheesy mass and examined it with a microspectroscope. To my great surprise, I again saw the same four-banded absorption spectrum which persisted even when the culture had been thoroughly washed with water. As the medium used for the cultivation of *B.subtilis* had been prepared from meat broth, a slight suspicion still lingered in my mind that there might be a connexion between the derivatives of haemoglobin in the culture medium and the pigment with the four-banded absorption spectrum.This suspicion could have been easily dispelled by examining fresh cultures of *B. subtilis* prepared without meat broth. However, before such a culture was obtained, I had the opportunity of examining with the microspectroscope a small lump of fresh baker's yeast compressed between two slides and was again surprised to find the characteristic four-banded absorption spectrum. This observation, together with that made on *Galleria*, clearly showed that this pigment which gave a four-banded absorption spectrum was completely independent of haemoglobin and its derivatives. At that time I had no knowledge of the main property of cytochrome, namely its reversible oxidation and reduction . . .

One day, while I was examining a suspension of yeast freshly prepared from a few bits of compressed yeast shaken vigorously with a little water in a test-tube, I failed to find the characteristic four-banded absorption spectrum, but before I had time to remove the suspension from the field of vision of the microspectroscope, the four absorption bands suddenly reappeared. This experiment was repeated time after time and always with the same result: the absorption bands disappeared on shaking the suspension with air and reappeared within a few seconds on standing.

I must admit that this first visual perception of an intracellular respiratory process was one of the most impressive spectacles I have witnessed in the course of my work. Now I had no doubt that cytochrome is not only widely distributed in nature and completely independent of haemoglobin but that it is an intracellular respiratory pigment which is much more important than haemoglobin. On the other hand, the nature of the pigment showing four distinct absorption bands when it was in the reduced state, but none when it was oxidised, still remained very obscure. Although I had by this time a first-hand knowledge of the absorption spectra of haemoglobin and of all its derivatives, I could not find among them a compound which in the reduced state showed four characteristic absorption bands (a–d) occupying the following approximate positions: a, 604 nm; b, 564 nm; c, 550 nm and d, 521 nm.

(from D. Keilin, *The History of Cell Respiration and Cytochrome*, Cambridge University Press, 1966)

If you take some of the yeast provided for the experiment in section 15.6.1, shake it with water in a test-tube, and examine it by holding it between your spectroscope and a bright light source, such as a table- or desk-lamp, you may be able to repeat Keilin's observations. If the yeast has been inactive a long time, it may be difficult to see the bands, but you should be successful with yeast that has been 'freshened' as described earlier. Alternatively, try with a piece of fresh baker's yeast as Keilin describes.

Shake the suspension, examine it, leave it to stand for a few minutes and re-examine.

What changes do you observe?

The absorption bands should disappear with shaking, reappear on standing.

What conclusions can you draw?

Keilin's conclusion was that the observation indicated that a reversible oxidation and reduction of an intracellular substance, cytochrome, was

occurring by reaction with the oxygen of the air.

Does this follow from your experiment?

No, because the substance might be reacting with other compounds of the air, like nitrogen, for instance.

How could you eliminate this possibility?

By repeating the experiment in the presence of different gases, such as pure oxygen, pure nitrogen, etc.

15.7.2 Oxidative phosphorylation

oxidative phosphorylation

The reaction sequence which goes from NAD by way of the cytochromes to molecular oxygen, then, is the cellular system of oxidation. Sending the hydrogen atoms down a series of carriers on their way to oxygen enables the available energy to be tapped off, so we may expect to find that their progress down the line is coupled with the production of ATP. This is indeed the case and, for oxidation to take place via the transport chain, it is necessary for ADP and phosphate to be present. During oxidation these substances are steadily used up, and ATP formed. When all the ADP or phosphate is finished, oxidation comes to a halt. The coupling mechanism is thus rather like the linkage between the engine of a car and its wheels, by way of the gears. Oxidation causes the engine to turn, and this in turn spins the wheels and forms ATP. But, if we stop the wheels from turning by putting on the brake or by removing the ADP supply, the engine stalls and oxidation ceases. In a car, we can prevent this stalling by uncoupling the engine from the wheels and letting it tick over freely by putting the gears in neutral. The cell too has such a mechanism for uncoupling oxidation from phosphorylation, which we shall discuss later in Unit 16 when we come to consider how the cell regulates its activities. But normally, the two are firmly linked.

If a quantitative study is made of the amount of ADP and phosphate used, and ATP made, per pair of hydrogen atoms passed down the line, it is found that three molecules of ADP disappear and three of ATP are formed during the transport of hydrogen from one molecule of $NADH_2$ to oxygen. How the reactions of oxidation are coupled to those of ATP formed during the sequence is known only in outline. One possibility is that the hydrogen carrier combines with an as yet unknown intermediate, and is then oxidized, forming in the process an 'energy-rich' substance. The breakdown of this intermediate can then be coupled with the synthesis of ATP from ADP and phosphate by way of the type of phosphorylated intermediate discussed in section 15.4. The nature and number of the intervening intermediates can only be guessed at, and it is not known whether they are in fact the same intermediates at each of the three coupling points. But the problem of oxidative phosphorylation is being intensively researched at present, and it cannot be too long before many of the present mysteries will be cleared up.

The data presented here as white-page material are not sufficient for you to calculate the net yield of ATP per molecule of glucose oxidized, so unless you care to read the *black-page* calculation of p.135 of *The Chemistry of Life*, you will have to take it on trust that 38 molecules of ATP are synthesized from ADP and inorganic phosphate for each molecule of glucose completely oxidized to carbon dioxide and water. This then, is the overall 'profit' to the cell from glucose oxidation: 38 molecules of ATP which can be subsequently utilized in the performance of synthetic

reactions, such as protein synthesis, or in cellular activities, like muscular contraction or nervous conduction, both of which are 'energy-requiring processes. Let us once again emphasize the universality of the processes and mechanisms that we have been discussing. ATP synthesis, like the Krebs cycle and the glycolysis reactions, is a biochemical process which appears to be of practically universal significance, from micro-organisms to man, and from yeasts to oak trees. It is one of the great underlying generalizations of today's biology (of which you will hear more in Unit 21) that the biochemistry of such different organisms has a fundamental unity which is in many respects much more significant than the undoubted differences that do occur.

Now do SAQS 8–13.

15.7.3 Where glycolysis and oxidation occur within the cell

So far, we have discussed the reaction sequences of glycolysis, glucose oxidation and oxidative phosphorylation, without reference to the cellular structures described in Unit 14, rather as if these complex enzymic sequences were occurring in dilute solution in the test-tube, instead of in the vastly complex and ordered system of the cell. Such a pretence has been convenient from the point of view of presenting the material, but it is time to abandon it.

The intact cell—whether it be of yeast, muscle, liver or a plant—can, under the appropriate circumstances, oxidize glucose by way of the glycolytic sequence and Krebs cycle, and synthesize ATP. You will see some of these reactions in the TV programme of this Unit. Where in the cell, though, are they carried out? Do all parts of the cell carry equal concentrations of all the enzymes necessary for these reactions? You have already met a technique which might help answer this question in Unit 14.

Can you suggest what it might be?

Subcellular fractionation.

The technique of subcellular fractionation can be employed to discover where the enzyme functions associated with glycolysis, the Krebs cycle and ATP are located. You will recall that, using the centrifugation procedure, we can isolate a set of cell fractions containing purified nuclei, mitochondria, cell membranes. The residual material, after all the subcellular particles have been centrifuged down, is regarded as containing the cell cytoplasm—the soluble cell constituents. If we examine these fractions for the presence of enzymes known to be involved in glucose metabolism, we find that they are very far from being equally distributed amongst them.

All the enzymes involved in the oxidation of pyruvic acid by way of the Krebs cycle, including the dehydrogenases and carriers of the hydrogen transport chain, are present *only* within the mitochondria, and cannot be found anywhere else within the cell. On the other hand, the enzymes of glycolysis, from the initial enzymes which phosphorylate glucose through to those which generate pyruvic acid and (in animal cells) lactic dehydrogenase, are located in the cell cytoplasm and in no other fraction. Neither group of enzymes is present in the nuclei, or the ribosomes, or the cell membranes. A preparation of cell cytoplasm will react with glucose, and generate pyruvic acid and (if it is from an animal cell) lactic acid. A preparation of mitochondria will not react with glucose, but will react with pyruvic acid, generating carbon dioxide and (under appropriate conditions) synthesizing ATP from ADP, inorganic phosphate and a source of protons.

We thus see that cell functions are not uniformly distributed, but localized. Particular cell organelles are, in fact, highly specialized structurally to carry out particular reaction sequences. Such functional specialization is known as *compartmentation* (see Figure 13).

compartmentation

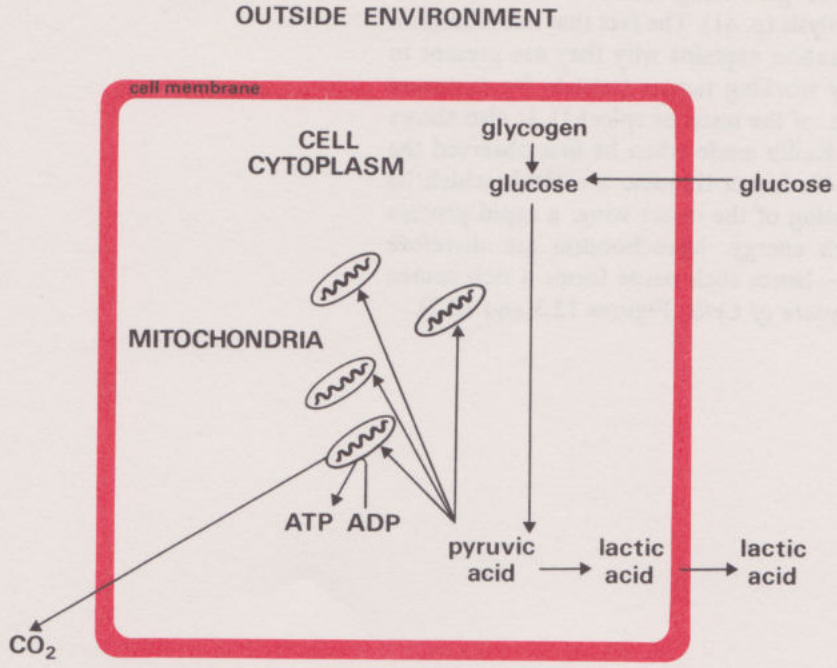


Figure 13 *Compartmentation of glucose metabolism within the cell.*

Thus, electron microscope pictures (as you have already seen in Unit 14) show that the mitochondria may be represented as in the cut-away drawing on p. 24 of *The Microstructure of Cells*. The structure is of two double membranes, an internal and an external one. The internal one is pulled into a series of folds (cristae) which run through the internal space of mitochondria, as you saw in the TV programme of Unit 14. Between the cristae, in the fluid-filled internal region of the mitochondria, are all the enzymes and intermediates of the Krebs cycle. Pyruvic acid, generated by glycolysis in the cell cytoplasm, enters the mitochondria and is there set upon by the Krebs cycle enzymes, which oxidize it to CO_2, and water. The CO_2 subsequently diffuses back out of the mitochondria and eventually out of the cell. Meanwhile, the $NADH_2$ generated during the oxidation remains within the mitochondria. The hydrogen transport chain is located in the internal membrane of the mitochondria and the cristae. The sequence of intermediate carriers, from $NADH_2$ through the cytochromes to molecular oxygen, are, it is believed, embedded within the lipid and protein-containing structure of the mitochondrial membrane in an ordered array, so that the spacial relationship of the individual molecules is as definite as that of ions in a crystal. Indeed, in one sense it is possible to regard the entire mitochondrion as a giant macromolecular complex of which the individual enzymes and carriers are no more than sub-components. The details of the exact array of the carriers, and their relationship to the supposed intermediates which link them to ATP production (see p. 49), and which are also supposed to be present in the inner membrane of the mitochondrion, are still far from certain, though they are being intensively studied at present. It may be that by the time you complete this Course it will be necessary to rewrite this entire section to accommodate new findings!

mitochondria

Meanwhile, we have provided in this section the answer to one of the questions we asked last week: 'is it possible to describe at the biochemical level the function and properties of the individual organelles of which the cell is composed? We can now say that for the mitochondrion the answer

is 'yes'. The mitochondrion has been aptly described as the 'power-house' of the cell, and its major role in cellular economy is the oxidative generation of ATP from ADP. Some 75 per cent of the total ATP production by the cell occurs within the mitochondrion, the remaining 25 per cent occurring in the cytoplasm by ATP-generating reactions such as those described as occurring during glycolysis (p. 41). The fact that mitochondria play this major role in ATP generation explains why they are present in much greater numbers in actively working tissues (muscle for instance) than in more quiescent tissues (e.g. of the testis or spleen†). It also shows what a fortunate choice of tissue Keilin made when he first observed the cytochromes, as described on p. 47. Insect thoracic muscles†, which he examined, are involved in the beating of the insect wing, a rapid process involving the utilization of much energy. Mitochondria are therefore numerous within the muscle cells—hence such tissue forms a rich source of cytochrome (see *The Microstructure of Cells*, Figures 12.3 and 12.7).

15.8 Green Plants and Photosynthesis

Study Comment

The synthesis of glucose by green plants is described in outline. The major points to grasp are that the process occurs in the chloroplasts, where again the relationship between structure and function can be seen, and that the biochemical mechanisms of photosynthesis are remarkably similar to those of glucose oxidation.

Green plants, like animals, require ATP for synthetic reactions and other cell activities; like animals, they obtain the bulk of this ATP by the oxidation of glucose and, like animals, they perform this reaction within the mitochondria present in the plant cell. The reaction routes of glycolysis and glucose oxidation are identical in both animals and plants, and to all intents and purposes the mitochondria of both are identical in appearance and function. The difference between plants and animals lies in the fact that in some plant cells, notably those of the green leaf, a system exists which can reverse the reactions of glucose oxidation, synthesizing glucose from CO_2 and water according to the overall reaction:

$$6\ CO_2 + 6\ H_2O \rightarrow C_6H_{12}O_6 + 6O_2 \quad \ldots\ldots\ldots (14)$$

This reaction is driven by energy arriving at the plant in the form of light energy from the sun, and it is the operation of this reaction which is known as *photosynthesis*. An outline of the operation of photosynthesis is provided on pp. 241–244 of *The Chemistry of Life*, which you should now read.

photosynthesis

Note that in this account we refer to the role of a coenzyme called NADP†, which you have not yet met in this Unit. As its name suggests, it is a close relative of NAD and, like NAD, is capable of being reduced, forming $NADPH_2$. We will meet it again in Unit 16.

Read the account of the chloroplast, the organelle responsible for carrying out photosynthesis, in pp. 45–48 of *The Microstructure of Cells*, and look at the photographs there.

chloroplast

We will return to the remarkable similarity between the mechanisms, enzymes and organelles involved in glucose oxidation and in photosynthesis in Unit 21. The similarity is, as we shall see, too striking to be coincidental, and has profound significance when we come to discuss the evolutionary origins of plants and animals.

For now, note that, in transferring our attention to the *synthesis* of glucose, we have also moved from a consideration of energy-*providing*, oxidative reactions, to energy-*utilizing*, *synthetic* reactions within the cell. It is to an examination of these, and of their control and regulation, that we proceed in more detail in Unit 16.

You should now try SAQS 16–21 *which relate to section* 15.8.

Unit 16

Section 1

16.1 Cell Synthesis

Study Comment

Energy-utilizing reactions are often associated with the synthesis of substances in the cell. Photosynthesis is just one example. Others are examined and discussed in this section—glucose in section 16.1.2, glycogen and starch in 16.1.3 and fat synthesis in 16.1.4. While it is not important to learn the details of any of these syntheses, it is important to realize the central role played by ATP in these reactions and the fact that to a large measure there is interconvertibility between the fats, proteins and carbohydrates stored in living systems.

16.1.1 The uses of ATP

In Unit 15 we looked at the group of reactions and structures within the cell associated with the synthesis of ATP from ADP. The ATP synthesis serves as a means of trapping the energy derived from oxidation (for instance, of glucose). ATP is in its turn used in a variety of anabolic reactions within the cell, involving the synthesis both of low-molecular weight substances such as purines, pyrimidines (Unit 10) and amino acids, and, more importantly perhaps, of complex molecules like polysaccharides, lipids, proteins and nucleic acids.

In addition, ATP is used for a number of functions involved with the maintenance of the internal structures and order of the cell. For instance, waste products of metabolism must be expelled from the cell and the body before they build up toxic concentrations. A notable example of this is in the metabolism of amino acids. Their breakdown ends with the carbon skeleton of the amino acid being converted into a form capable of being oxidized through the Krebs cycle, but the other constituent of amino acids is nitrogen, and the form in which amino acid breakdown tends to release it is as ammonia, NH_3. Ammonia is toxic to the cell in even quite low concentrations, and in man the bodily mechanisms for removing it involve converting it to a relatively non-toxic substance, urea, $CO(NH_2)_2$, which can be excreted in the urine. The synthesis of urea is an energy-requiring process which can be performed by enzymes contained in the cells of the liver. During the synthesis of urea, ATP is utilized, being broken down in the process to ADP once more. You will come across other examples of the use of ATP in maintenance of cell composition later in this Unit.

All these demands on the cell's ATP resources arise in the processes of preservation and maintenance. In addition demands arise from the cell's active roles, such as the synthesis and secretion of hormones in endocrine cells (see Unit 18), or the contraction of muscle cells. Within the cell of an active body tissue, brain or liver, for example, any molecule of ATP is likely to be broken down to ADP and converted to ATP again some thousands of times per hour. We begin this Unit by considering some of these synthetic reactions involving ATP.

16.1.2 Interrelations of synthesis and breakdown

Consider the photosynthetic production of glucose, discussed in section 15.8. What are the subsequent uses of this molecule? One is as a source of available energy for the plant cell itself, both in sunlight and subsequently in the dark when photosynthesis no longer operates. A second is that *all* the cells, in plants and animals, require glucose so as to generate ATP, even though only the chlorophyll-containing ones in green plants synthesize it from CO_2. So it is advantageous to the plant if more glucose is synthesized during daylight than is consumed immediately. The glucose molecule is not, however, accumulated as such, but is synthesized into the convenient macromolecular form in which the plant stores glucose.

It is the plant's reserves of starch which provide glucose for the animals which eat the plants. Humans lack the enzymes for cellulose breakdown, but possess those such as the salivary amylase you used in Experiment 15.3.1 which will break down starch. But it can happen, in the same way as with plants, that the intake of glucose in the animal diet is too great for immediate utilization, and a similar synthesis of a storage molecule occurs in animals too.

Glycogen is stored mainly in liver and muscle in animals, both of which have the enzyme systems for its synthesis. Other body tissues, such as brain, do not possess these enzymes and do not store glycogen in significant quantities.

When the metabolic demands on glucose are high, starch in the plant and glycogen in the animal are broken down to glucose once more and the glucose can enter the metabolic pathway leading to oxidation that we have already discussed. This breakdown involves an enzyme, which ruptures the linkages between glucose units in the molecule. The reaction requires inorganic phosphate, and the glucose units are released as molecules of glucose phosphate which, by way of other transformations, can undergo glycolysis.

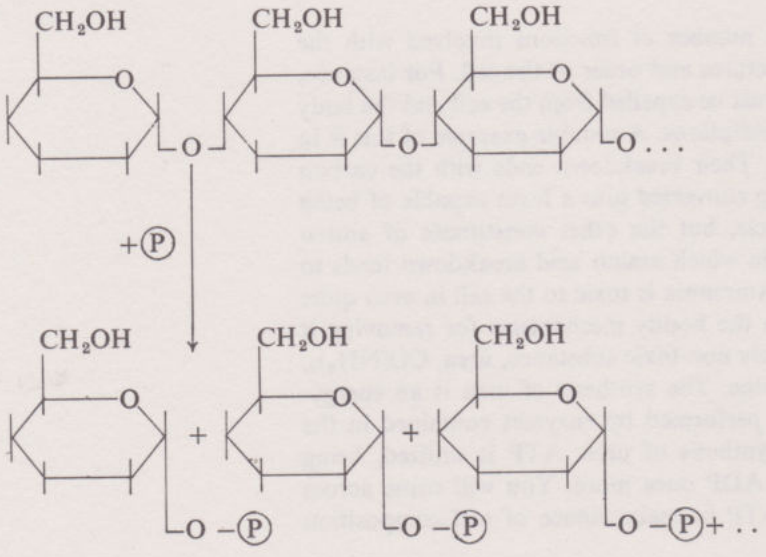


Figure 1

Some animal tissues, like the brain for example, do not have their own intracellular supply of glycogen and must rely on glucose taken from the circulating blood stream. A lowering of blood glucose, by uptake into the brain, results in a mobilization of liver glycogen; it is converted to glucose, passes into the blood and hence to the brain and other organs. On the

other hand, there is a limit to the amount of glycogen which the liver can accumulate in times of plenty—for instance, shortly after an adequate meal. When the liver glycogen stocks are full, glucose is converted by way of a complex sequence of reactions into fats, which act as a more long-term store of oxidizable, and hence ATP-generating, molecules. These fats are then laid down in various depots around the body, notably as a layer beneath the skin. It is this fat which, present in excessive quantities as a result of too great an intake of carbohydrates (and, to a lesser extent, fats) in the diet, results in the phenomenon, prevalent at least in the well-fed Western world, of obesity. The breakdown of the body fat, which provides an alternative source of oxidizable molecules to glucose, is accelerated in the body if the blood glucose levels, and the muscle and liver glycogen levels, fall. Hence the most successful way to slim, for normal people, is to lower their dietary carbohydrate intake. The synthesis, breakdown and inter-conversions of fats and carbohydrates all interrelate therefore, and you can read something of the mechanism (as black-page material) on pp. 147–149 of *The Chemistry of Life*. Note in particular the central role of the Krebs cycle in these interactions.

16.1.3 The synthesis of glycogen and starch

synthesis of glycogen

The polysaccharides, as we saw in the previous section, are broken down into monosaccharides by a hydrolytic reaction involving the enzyme phosphorylase. It might be expected, therefore, that the *synthesis* of glycogen or starch would proceed by the reversal of the route of their breakdown and, indeed, this was the first hypothesis made by biochemists working in this area. They were, however, to be proved wrong. To see how and why, and to study the mechanisms of glycogen synthesis (the synthesis of starch follows a similar pathway), read now *The Chemistry of Life*, from the top of p. 152 to p. 154.

16.1.4 Other synthetic reactions

The synthesis of glycogen is an example of how, in the cell, it is simpler to destroy than to build. An even clearer indication of this is provided if we contrast the synthesis and the breakdown of fats within the body. Those of you with the time to study the details of this contrast, should compare fat oxidation, discussed on pp. 138–143 of *The Chemistry of Life*, with fat synthesis, dealt with in pp. 155–159 (black-page material).

These pathways are shown, too, in the 'metabolic chart', Figure 7 of Unit 15, attached to the inside back cover of this book.

For our present purposes, the major lessons of these contrasts are that:

1 whilst fat breakdown occurs in a step-wise series of reactions analogous to those of glycolysis and glucose oxidation, the synthetic route involves a more complex pathway, whose enzymes seem to be bound together (like the mitochondrial hydrogen carriers for $NADH_2$ oxidation discussed in Unit 15) as a 'multi-enzyme complex' located in the cell cytoplasm; and

2 whilst fat *oxidation*, like glucose oxidation, results in the generation of the reduced co-factor $NADH_2$, fat *synthesis* involves the related substance NADP (which you will recall having met as a coenzyme of photosynthesis). During fat synthesis, which is, as the reverse of oxidation, a *reductive* sequence, reduced $NADPH_2$ is *oxidized* to NADP.

One of the purposes of stressing the relationship between synthetic and degradative reactions like this is to prepare, from the biochemical point of view, to deal with the more complex, and more exciting, problems of protein and nucleic acid synthesis, which are crucial to our understanding of cell maintenance and growth, replication and genetic continuity. These syntheses, and their genetic significance, which form a part of the relatively recent area of science known as *molecular biology*, will be the major theme of Unit 17.

Now do SAQS 1–8, *which relate to section* 16.1, *and to sections* 15.6 *and* 15.7 *in Unit* 15.

Section 2

16.2 ATP Utilization in the Active Cell: Muscular Contraction

Study Comment

Synthetic reactions are only one of several types of reactions which use up ATP in living systems. A second type, in which a cell performs mechanical work, is examined and discussed in this section. The importance of ATP in this system should be understood. The relationship between structure and function is well illustrated in striated muscle and should be noted. Do not attempt to memorize the detailed fine structure of muscle cells.

So far we have been concerned with the utilization of ATP in performing chemical work in the maintenance of the cell's macromolecular components. We can now turn to an example of the way in which the breakdown of ATP can be coupled to the performance of mechanical work. No better example of this can be found than the mechanism of muscular contraction, the details of which provide a fascinating demonstration of the way in which the specialization of individual cells and their molecular components provide a structure admirably fitted to the function of the performance of work by the cell upon the external environment.

muscular contraction

Look again at the electron micrographs of striated muscle shown in *The Microstructure Cells*, pp. 38–43, and then read the account of muscle contraction and the role of ATP given in *The Chemistry of Life*, pp. 220–225.

16.3 Control and Regulation in the Cell

Study Comment

Various ways in which the biochemical activity of cells is regulated and controlled are discussed and the whole topic is set in the larger framework of self-regulating systems both living and non-living. You should learn the major control systems found in cells and the differences between them.

In Unit 14, we suggested that the history of the biologist's approach to living organisms involved the asking of a series of questions of increasing complexity.

Do you recall these questions?

They were related to: (1) the composition of the organism at a chemical and structural level; (2) the relationships between the chemicals: how they are interconverted; (3) the control and regulation of these interconversions so as to maintain organized structure and activity; (4) the distinction between any one cell of a given tissue or organ; and, the others, the relationship between structure and function.

Unit 14 dealt with question (1) and introduced a discussion of (4) that will be taken up more fully in Unit 18. In Unit 15, and so far in this Unit, we have been considering aspects of question (2); we have been concerned to describe the metabolic reactions occurring within the cell in terms of a *traffic in energy*; reactions were classified as energy-yielding and energy-requiring, and we considered the relationships between ATP production and ATP breakdown. On the basis of what you have learned so far, it is now possible to turn to an approach to question (3); the rest of this Unit will be devoted to discussing it. From one point of view, what you will be discovering in Unit 17 and in Unit 18, when we ascend the scale from cells to organisms, can be analysed into the same themes of control, organization, and regulation.

traffic in energy

The point is that the cell, as should now be clear, is very far from being a random bag of chemicals and structures. The high degree of order and regulation necessary to conduct the organized reaction sequence of oxidative phosphorylation in the mitochondrion or photosynthesis in the chloroplast, for example, should have made this obvious. How does the cell 'know' for instance, how much glucose to oxidize and how much to convert to glycogen at any point, or how many of any given class of enzyme molecule to synthesize? In answering questions of this sort, we must abandon thinking in terms of a 'traffic in energy' but instead think in terms of a new concept, that of *information*. This concept is one that can be given a precise meaning. It is possible to calculate the 'amount of information' contained in a communication in terms of a unit known as a 'bit' (which is short for a *b*inary dig*it*), and this calculation is the basis for theories and problems in communications and electronics which do not concern us further here. 'Information theory', which is closely associated with the branch of science which has been given the name 'cybernetics', was developed in the 1940s and 1950s by the American mathematician Norbert Weiner, although an early forerunner was the nineteenth-century British scientist Charles Babbage (see also *The Roots of Present-Day Science*). The revolution in scientific thinking following the introduction of these concepts has undoubtedly made possible not only new analyses of the behaviour of biological systems, but also the development of computers, sophisticated machine tools and other processes for which the ugly name 'automation' has been coined. The needs of the communication industry since the Second World War have led to a great development of cybernetics, but here we need only refer to some of the more basic concepts and we will treat these in a non-mathematical, descriptive way—you

information

cybernetics

will be able to hear more concerning these topics in the radio programme of Unit 18.

To approach the problem of control and regulation, it is appropriate to consider cellular problems in terms of a 'traffic in information'. These ideas are explained more fully, in *The Chemistry of Life*, Chapter 10, pp. 187–201, which you should now read. (The role of hormones, dealt with in the second part of that chapter, will be discussed from a more 'whole-organism' and less 'biochemical' point of view in Unit 18.)

In reading this chapter you may like to note the following points of commentary upon it.

p. 192, 'cyt a_3'. This refers to the terminal cytochrome in the chain of carriers from $NADH_2$ to oxygen, one of those referred to in Unit 15, section 15.7.

p. 194, 'thyroxine'. The activity of this hormone will be dealt with again in Unit 18.

p. 197. The discussion of the synthesis of enzymes will be dealt with in Unit 17 next week.

lysosomes

p. 198, 'lysosomes'. We have not yet discussed the role of these organelles. You can see an electron micrograph picture of them in *The Chemistry of Life*, plate 4, facing p. 136. Biochemically, the lysosome, discovered in 1953, is an extremely interesting organelle, which provided a solution to a long-standing problem. The cell, when broken by homogenization, contains a variety of enzymes which are capable of destroying many of the key substances within the cell. DNase, RNase, proteases, phosphatases will hydrolyse nucleic acids, proteins and phosphates respectively. The cell will easily succumb to a purified preparation of any of them. How are they kept in check by the living cell? The answer is that they are all contained within the lysosomal particle, and the cell is protected from them by the lysosomal membrane behind which they are caged. All these enzymes have their pH optimum in the acid range. If the cell is damaged, or dies, the lysosomal membrane bursts as a result of changed osmotic pressures and the enzymes are released into the cytoplasm and begin to function. The products themselves tend to be acid, and the pH of the damaged cell is lowered, increasing the enzyme activity and establishing a positive feedback loop, bringing about a total cell dissolution in short order.

The lysosomes, therefore, function as a party of cellular scavengers, harmless to the healthy, but quickly destroying sick or injured cells. Sometimes this destruction can be functional, as in the developing frog, where the lysosomes in the tadpole's tail cells perform a vital role in digesting the tail, helping on the subsequent stages in development. These are not the lysosomes only functions. In addition, the enzymes within the lysosome are capable of breaking down or inactivating certain toxic substances entering a healthy cell. The substances are taken up into the lysosomes and rendered relatively harmless. Thus as well as destroying the sick, the lysosomes help keep the fit cells healthy. You will see more of this discussed in the TV programme of this Unit.

p. 198. DNA and the genetic code will be discussed in Unit 17.

active transport

p. 199. The capacity of the external cell membrane to selectively permit some ions to enter the cell and others to leave is remarkable in that the cell will take up preferred ions from the surrounding fluid, and expel others from within the cell. The ions may be accumulated inside the cell until their concentration within it is many times greater than outside. This phenomenon is known as *active transport*. It is not confined to sodium and potassium but appears to extend to practically all other substances which enter the cell. Thus a slice of brain or liver tissue, incubated in an appro-

priate medium, will accumulate amino acids until the intracellular concentration is 10 or 20 times that outside the cell. That such processes are energy-dependent can be shown with the following neat experiment with red blood cells, which normally maintain high internal potassium and low internal sodium concentrations. If the cells are collected from blood and kept in the cold (at about 4° C instead of the normal 37° C), sodium enters the cell and potassium leaves until the concentrations on both sides of the cell membrane equilibrate. Placing the cells in a warm solution with glucose as an energy source now results in a rapid decrease in internal sodium and increase in potassium. Addition of iodoacetic acid to the medium prevents this change. By a combination of experimental tricks it is possible to rupture the red blood cells in a dilute solution. All the internal cell constituents, including the enzymes responsible for glucose metabolism, leak out. Replacing the ruptured cells in a medium of appropriate ionic strength will cause the membrane to reseal, producing a preparation of empty red cell 'ghosts'. These will not maintain high internal potassium concentrations. But if the 'ghosts' are reconstituted in a medium to which a small quantity of ATP has been added, they reseal with a certain amount of ATP *inside* them. Placing them in a warm medium containing sodium and potassium at appropriate concentrations but without glucose will now result in potassium being accumulated and sodium extruded once more, and, during this process, the cellular ATP is converted to ADP. There is some evidence that the mechanism of active transport in red blood cells and other tissues involves the reaction of ATP with protein constituents of the cell membrane, thus altering the membrane configuration so as to permit one ion to enter and the other to leave. The whole process is of particular significance, as it forms, as well as an apparently universal mechanism of cell ion selectivity, the basis for the propagation of the nerve impulse.

p. 201. Whilst the explanation given is one possible way of accounting for the Pasteur effect, there are alternatives, and at this stage in research the correct mechanism is still uncertain.

You should now be in a position to attempt SAQS 9–24 *which relate to material from section* 16.3 *and Unit* 15.

16.4 Assembling the Cell

Study Comment (*sections* 16.4, 16.5 *and* 16,6)

Some of the philosophical implications involved in and arising from the study of cell biology are explored in these three sections. It is important to be clear about these because they are the key to asking sensible questions and so the key to understanding not only the answers to particular questions but also the point and purpose of cell biology.

From what you have read so far in this Unit, you will understand that we can account for many features of cell behaviour and cell properties in terms of the ideas of control and regulatory processes of information flow between different parts of the cell. Of all these controlling features, the most striking, perhaps, is the relationship between structure and function of the individual cell organelles. Nucleus, mitochondria, lysosomes, ribosomes and cell membrane all have their parts to play in keeping the cell in order (though you will not learn in detail of the role of two of these, nucleus and ribosomes, until Unit 17). These organelles have a complex structure of their own, as we have seen in the case of the mitochondrion. The question must then be asked, 'how are these organelles themselves synthesized and held together?' Have we at last asked a question to which no answer can be given? The evidence we can offer on this point is only suggestive and far from complete, because it is only in very recent years that research on this sort of problem has begun to be carried out. All that is possible here is to assemble some of these ideas and results and indicate where they point.

If you recall what was said about protein structure in Units 13 and 14, you will remember that proteins have, as well as primary and secondary structures, tertiary structures too; the folds and coils into which they are bent when seen in three-dimensional space. It is becoming clear that this tertiary structure is not a chance form into which the protein happens to fall, but that when the protein chain is synthesized (by pathways you will discover in the next Unit) it has only a very limited range of configurations that it can possibly take up under the ionic conditions of the cell. This tertiary configuration is a stable one, governed by kinetic and ionic considerations that are not yet fully understood. However, it is clear that within the cell the conformation of the individual protein molecule is specified by its micro-environment.

This is true not only for individual proteins, but for assemblages of proteins as well. The enzyme system responsible for oxidizing and removing carbon dioxide from pyruvic acid (reaction 6 of Unit 15) is a complex one involving several individual reactions each catalysed by a separate enzyme. These enzyme proteins can be individually separated and purified. But in the cell, the complex group of protein molecules are in close association and indeed are bound to one another as a *quaternary* complex. It is so big that electron micrographs of the individual enzyme complex can be made (Fig. 2). One intriguing feature of this complex is that, if the individual purified enzymes are mixed together, *in vitro*, in a solution containing the right concentrations of other ions, the complex will reform; it will assemble itself from its component molecules. This self-assembly is governed by similar kinetic considerations to those that affect the individual proteins.

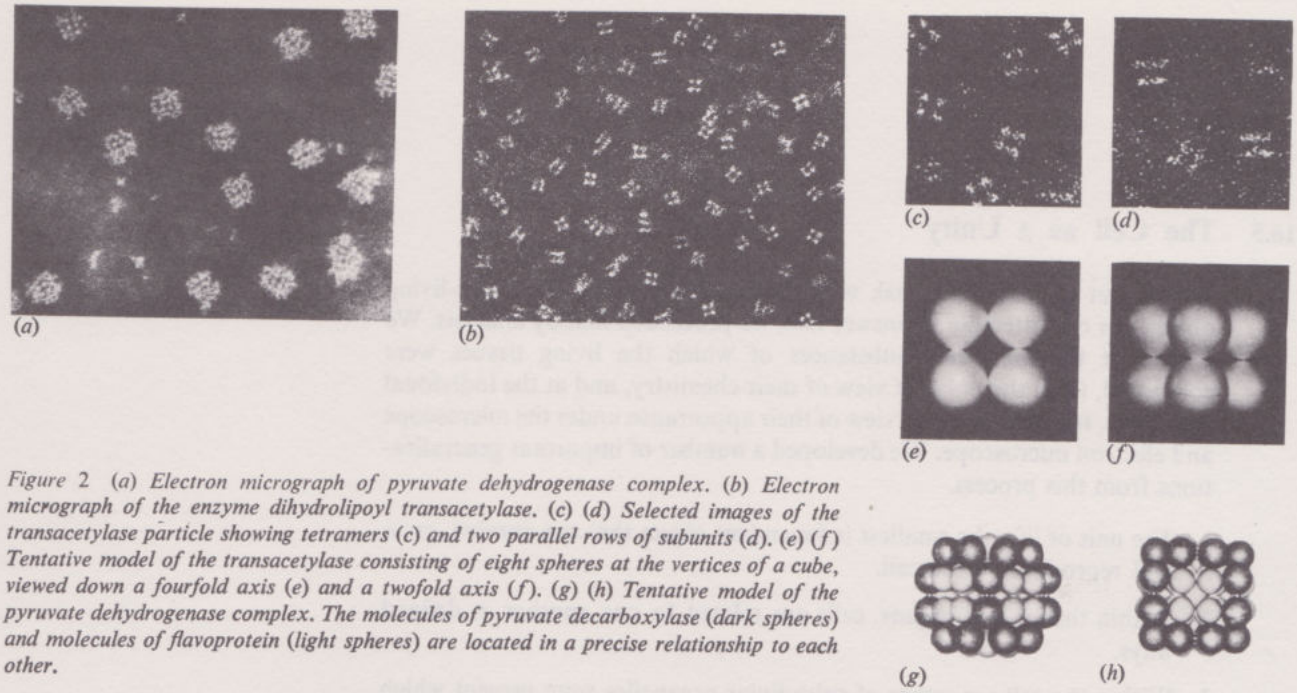


Figure 2 (a) Electron micrograph of pyruvate dehydrogenase complex. (b) Electron micrograph of the enzyme dihydrolipoyl transacetylase. (c) (d) Selected images of the transacetylase particle showing tetramers (c) and two parallel rows of subunits (d). (e) (f) Tentative model of the transacetylase consisting of eight spheres at the vertices of a cube, viewed down a fourfold axis (e) and a twofold axis (f). (g) (h) Tentative model of the pyruvate dehydrogenase complex. The molecules of pyruvate decarboxylase (dark spheres) and molecules of flavoprotein (light spheres) are located in a precise relationship to each other.

More impressive examples of the same phenomenon also exist. The ribosome, an organelle whose function will be explained in Unit 17, consists of two sub-units each containing thirty or more individual proteins and a large amount of RNA (you can see a picture of the ribosome on p. 171 of *The Chemistry of Life* and in Unit 14). In 1969, one of these sub-units was broken up into its individual proteins by some Japanese research workers in the USA. The individual proteins were then mixed together again in appropriate concentrations in a solution containing some RNA, and surprisingly and impressively, the ribosomal sub-unit was found to reassemble itself from these components in an *in vitro* system, and without the need for even a source of energy like ATP. Once again, presumably, the complex interactions of the individual molecules must be assumed to be determined by kinetic and ionic considerations which direct the form of the stable complex.

Similar self-assembly of membrane-like structures can be shown to occur in simple model systems in which molecules of lipids and proteins are mixed and introduced into an appropriate solution. The lipoprotein complexes which are produced can take the form of micelles (Unit 14) which under the electron microscope have the characteristic appearance of the 'double membrane' structure shown so clearly in Figure 3.2 of *The Microstructure of Cells.*

Not only subcellular organelles, but whole cells, may perhaps be able to assemble themselves in this way. In 1970, workers in the USA were able to show that if the cells of a large unicellular organism like *Amoeba* (see Unit 18 TV programme) were carefully taken apart, and then the nuclei, cell membranes and cytoplasm collected and remixed in appropriate proportions, functional cells developed once again. In the next Unit you will learn of similar experiments with a virus.

All this evidence is suggestive rather than conclusive, and these phenomena cannot yet be fully analysed in the molecular terms we have used in this Course. Nonetheless, they are indicative of the degree to which the cell can now be described as something very different from the random bag of chemicals or the formless 'protoplasm' of the early microscopists, and of how, in the contemporary development of cell biology, many of the hitherto inexplicable mysteries of life are beginning to fall into place.

16.5 The Cell as a Unity

We set out in Unit 14 to ask what distinguishes living from non-living objects. In our attempts to answer this, we proceeded first by analysis. We looked at the individual substances of which the living tissues were composed, from the point of view of their chemistry, and at the individual structures, from the point of view of their appearance under the microscope and electron microscope. We developed a number of important generalizations from this process.

1 The unit of life, the smallest independent object that can survive, grow and reproduce, is the cell.

2 Within tissues and organs, cells are related to one another in defined ways.

3 Within the cell a number of subcellular organelles were present which also have defined compositions, structures and relations with one another.

4 Although often complex, both the low-molecular-weight chemicals and the giant molecules in the cell may be analysed and synthesized by extensions of existing chemical techniques; there is apparently nothing uniquely living about them.

On this basis, we proceeded to ask the question: was there something uniquely living about the chemical interactions of these components and structures, their synthesis, breakdown or transformation? Again we showed that this was not the case, for all such transformations are amenable to the analysis of reaction mechanisms (although treated in these Units at an elementary level) of the type offered for simpler reactions in Units 9, 10 and 11.

Two further major concepts are important for elucidating these areas of cell biology. First, that of the existence of a class of protein molecules whose structures enabled them to function as highly specific chemical catalysts, thus governing the individual cell reactions. These are enzymes. Second, that of the presence of a procedure within the cell for associating endothermic and exothermic reactions by way of the linking molecule ATP, synthesized during one process from ADP and inorganic phosphate, and broken down to ADP and inorganic phosphate again in the other. These ideas enable us to demonstrate in general terms that the cell can synthesize its own constituents. (The one major constituent, protein, which we did not deal with, is described in Unit 17.) These syntheses use as their ultimate energy source, solar radiation, utilized by plants in photosynthesis of glucose. This is the area of biology that we referred to in Unit 14 as kinetics.

If life does not reside in enzyme mechanisms or ATP, could it reside in the capacity of organisms to control these chemical mechanisms and so achieve an ordered cell structure? The major generalizations of information and control, and the ideas of rate-limiting reaction sequences, of enzyme induction and of structural barriers to indiscriminate reactions, show that these properties of living organisms too are approachable using the same analytical techniques as have applicability outside living biological systems.

Finally, it was suggested (though not strictly proved) that such factors were able to account also for the self-assembly of at least some cell structures from their molecular components.

Where then is the difference between life and non-life? A cell is clearly living. Have we explained away all that there is to say about life by subjecting it to analysis and experiment? Such questions have obviously considerable philosophical interest. It has been argued that life is 'nothing but' an assembly of molecules and chemical reactions. This position has been described as *reductionism*. On the other hand, it has been claimed that there is something about living systems which 'can never' be explained by accounting for them in terms of molecular or structural interactions. Such a viewpoint is sometimes referred to as *holism*—viewing the cell and the organism as a whole. This must not be confused with the 'vitalism' to which we referred in Unit 14. Vitalism implies that there are some special properties of living organisms which cannot be explained by the methods of science and the 'natural laws' which have been developed from a study of non-living matter. Holism would allow the existence of scientific 'laws' to describe cells, or organisms, but would not permit their derivation from the properties of the molecules of which the cells are composed.

reductionism

holism

The descriptions provided in these Units do not favour either of these extreme positions (although here we are stating an extrapolation based on the type of data presented here which is, strictly speaking, unprovable). Rather, we wish to suggest that it is more a question of just *what* one is trying to explain. In this Course, we have proceeded up the scale of size and complexity from atomic nuclei and electrons to atoms, molecules and cells. Whilst it is doubtless possible to specify all the individual atoms of the cell and their electron states, for the purposes of providing a meaningful description of the cell, it is not useful to do so. Whilst some progress has been made in analysing the detailed kinetics and chemical mechanisms of some enzymic reactions, once again such a degree of intimate detail is generally not likely to be helpful in a discussion of the control of cellular reaction pathways, or the role of the mitochondria or other organelles within the cell.

In general, we can classify different levels of analysis of life, ranging from the atomic to the cellular and the organismic. Such levels are referred to as hierarchies and we can draw them as shown in Figure 3.

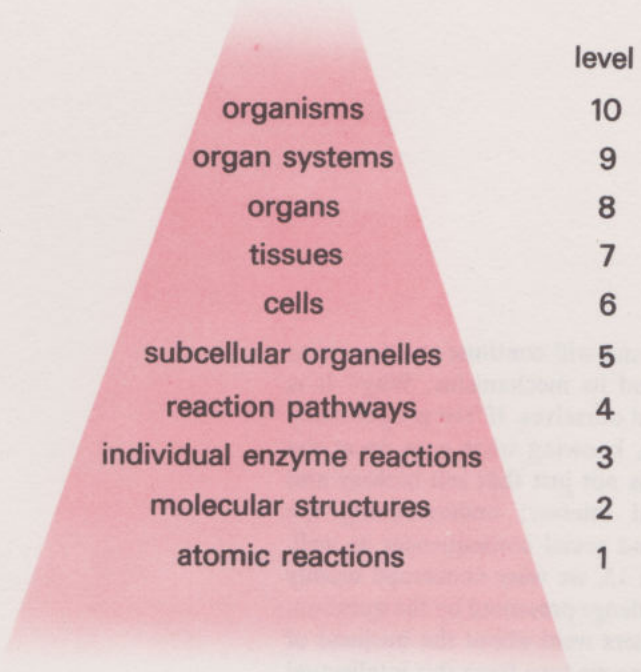


Figure 3

(Note: there are subatomic hierarchical levels that we have not yet described; you will meet them towards the end of this Course. The level

of organism you will meet in Unit 18; and there are levels above the organism (e.g. populations and species) that are discussed in Units 19–21.)

Generally speaking, reducing a discussion of the properties of objects at one level in the hierarchy to those at the level below increases the precision with which we can talk about them, but at the same time decreases what we may describe as the *meaningfulness* and *usefulness* of the description for dealing with interactions either between objects at the same hierarchical level or at the next hierarchical level up. On the other hand, it is frequently only possible to make an experiment about objects at one hierarchical level by approaching them from below. To understand the cell at level 6 we had to take it to pieces and deal with it at levels 5, 4, 3, and 2. Some of the experiments we have described in detail in Unit 15, and this Unit, have demonstrated this taking of the cell to pieces in order to put it together again at the next hierarchical level up. It is this situation which makes it difficult to answer the question 'what is life?' without seeming to be trivial. Thinking, writing or reading this Unit, making love or hearing music, are phenomena which can in principle be described in terms of cell interactions, or atomic interactions, but only if we realize that what we are doing when we try to make descriptions 'across hierarchical levels', in this way, has its limitations as well as its advantages. For some cross-hierarchical descriptions the limitations might far outweigh any explanatory advantage that could possibly accrue.

Section 6

16.6 Why Study the Cell?

We have assumed in Units 14, 15 and 16, and will continue to do so, that it is worthwile to try to study the cell and its mechanisms. Why? It is clearly of intellectual interest to understand ourselves. If 'the proper study of mankind is man', as has been claimed, knowing what goes on at the cellular level is part of that study. But it is not just that cell biology and biochemistry are of abiding intellectual interest; understanding the mechanism of life potentially has profound social consequences as well. In the radio programmes of Units 14 and 15, we were concerned mainly to explain the nature of the intellectual challenge presented by the question, 'what is life', and how individual researchers went about the business of answering it. In this Unit's radio programme we turn from this intellectual challenge to an examination of the uses and abuses of the new biology of the 1970s. It is in the light of these possibilities that we must assess the significance and potential of the biology taught in these Units and researched in the laboratories of this country and throughout the world.

16.7 Summary of Units 15 and 16

1 Within all the cells of the body there is a continuous and rapid process of breakdown and resynthesis of the molecules of which the cells are composed. This phenomenon is known as the 'dynamic state of body constituents'. (15.2)

2 The synthesis and breakdown proceed by a series of stepwise chemical reactions, each of which is catalysed by an *enzyme*. Enzymes are protein molecules which function by forming intermediate complexes with the substances they react with (known as *substrates*). Enzyme reactions can be speeded up by *coenzymes*, slowed by *inhibitors*. An experiment on enzyme reactions was performed. (15.3)

3 Some reactions within the cell are exothermic, some endothermic. In general, synthetic reactions like the production of protein from amino acids are endothermic. To 'drive' the endothermic reactions, the molecules involved in the synthesis are activated, often by phosphorylating (adding phosphate to) them. This phosphorylation is performed at the expense of the substance *adenosine triphosphate* (*ATP* for short). In its turn, ATP is synthesized from *adenosine diphosphate* (*ADP*) and phosphate during exothermic reactions such as the oxidation of glucose. (15.4)

4 A number of ways in which cellular reactions can be unravelled were described. (15.5)

5 The major energy-yielding (ATP-synthesizing) pathway of the cell, that of glucose oxidation, was described, with an exercise on experiments concerning glucose breakdown during muscle contraction. Both *glycolysis* and the *Krebs cycle* were discussed in outline. (15.6)

6 The oxidation of the reduced coenzyme $NADH_2$, generated during the breakdown of glucose, was described. The coupling of this oxidation with ATP synthesis from ADP was discussed. (15.7)

7 The major subcellular sites of glycolysis (the cytoplasm) and glucose oxidation (the mitochondria) were analysed. (15.7.3)

8 The parellel synthetic mechanism of *photosynthesis* in the green plant, by which glucose synthesis from CO_2 occurs, was discussed together with the role of the chloroplast. (15.8)

9 Attention then shifted to the major pathways which utilize ATP; *glycogen synthesis* was the example used. (16.1)

10 Another major system which utilizes ATP is one not of synthesis but of cell activity; the example described was that of *muscular contraction*. (16.2)

11 At this point, we turned from a discussion of the properties of the cell analysed in terms of a *traffic in energy* (ATP) to a discussion of the means by which this traffic is *controlled* within the cell. We considered another way of analysing cell properties, in terms of *traffic in information*. Various control techniques, *feedback loops*, *product inhibition*, *rate limiting steps* and *structural control mechanisms* were described, with examples. (16.3)

12 Finally, we considered the questions which lie at the back of our endeavour to understand the cell. To what extent is it possible to meaningfully describe living processes in terms of the behaviour of individual molecules, and to what extent is it necessary to introduce supramolecular concepts of organization? The role of hierarchies, and the meaning of the terms *holism* and *reductionism* (16.4, 5 and 6) were described.

Parallel and Background Reading for Units 15 and 16

Chapters appropriate to Units 15 and 16 are shown in brackets after each title.

Note: these are *alternatives*, and generally deal with each topic in much greater depth than is done in these Units, and with a much heavier load of factual material.

For full details of each title see Unit 14, p. 38.

M. J. Berril, *Biology in Action* (Chapter 4).

P. B. Weisz, *et al.*, *The Science of Biology* (Chapters 5, 18, 19).

S. D. Gerking, *Biological Systems* (Chapters 8, 14).

A. G. Loewy and P. Siekevitz, *Cell Structure and Function* (Chapters 2, 3, 10, 11, 12, 13, 14, 18).

J. A. Ramsay, *The Experimental Basis of Modern Biology* (Chapters 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20).

H. R. Mahler and E. V. Cordes, *Basic Biological Chemistry* (Chapters 6, 7, 8, 11, 12, 14, 15).

E. E. Conn and P. K. Stumpf, *Outlines of Biochemistry* (Chapters 6, 7, 8, 9, 11, 12, 16, 17, 21).

Acknowledgements

Grateful acknowledgement is made to the following sources for material used in these Units:

TEXT

Cambridge University Press for David Keilin, *The History of Cell Respiration and Cytochrome*; Penguin Books Limited for Steven Rose, *The Chemistry of Life*.

ILLUSTRATIONS

Professor Lester J. Reed for Fig. 2a–h.

Appendix 1 (White)

Glossary

A-BAND One of the characteristic striations seen in voluntary muscle at high magnifications. See *The Microstructure of Cells* for photograph.

ACTIN One of the muscle proteins involved in the contractile process.

CREATINE, CREATINE PHOSPHATE Molecules which can readily exchange phosphate with the terminal phosphate group of ATP; function as an 'energy-bank'.

DEHYDROGENASE Enzyme which oxidizes its substrate by removing hydrogen from the molecule, generally passing it to a coenzyme such as NAD.

HAEMOGLOBIN Iron-containing protein present in blood and responsible for its characteristic red colour. Combines reversibly with oxygen.

HYDROGEN CARRIER Substance present in the mitochondria which is reversibly oxidized and reduced during biological oxidations.

MANOMETRY Method of measuring rate of metabolism of a substrate participating in biological oxidations.

MYOSIN One of the muscle proteins involved in the contractile process.

NAD, NADP Abbreviations for two coenzymes, *n*icotinanide *a*denine *d*inucleotide and *n*icotinanide *a*denine *d*inucleotide *p*hosphate, which undergo reversible oxidation and reduction during biological oxidations and synthetic reactions.

OXIDASE Enzyme which catalyses the direct oxidation of substrate by addition of oxygen originally derived from the atmosphere.

RANEY NICKEL Particular form of the metal nickel which is catalytically active in oxidation/reduction reactions.

SCINTILLATION COUNTER Device for measuring the amount of a radioactive isotope present in a sample.

SPECTROPHOTOMETER Instrument which measures the absorption of light at particular wavelengths in both the ultraviolet and visible range, and used in a manner similar to the colorimeter in enzyme determinations.

Z-BAND One of the characteristic striations seen in voluntary muscle at high magnifications. See *The Microstructure of Cells* for photograph.

Self-Assessment Questions

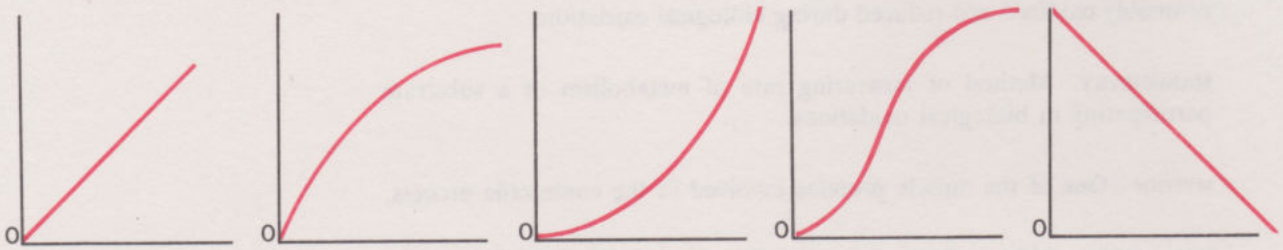
Section 15.3

Question 1 (*Objective 2 (ii)*)

Which of the following is the best evidence for the 'lock and key' theory of enzyme action?

(i) All isolated enzymes have been identified as proteins.
(ii) Compounds similar in structure to the substrate inhibit the reaction.
(iii) Enzymes are found in living organisms and speed up certain reactions.
(iv) Enzymes speed up reactions by definite amounts.
(v) Enzymes determine the direction of a reaction.

The graphs drawn below, and Questions 2 *and* 3 *refer to an enzyme-catalysed reaction.*



Question 2 (*Objective 2 (ii)*)

If an enzyme-catalysed reaction requires molecular contact between enzyme and substrate, then which figure best represents the relation between the rate of a reaction and substrate concentration? (Let the horizontal axis represent substrate concentration and the vertical axis represent reaction rate.)

Question 3 (*Objective 2 (ii)*)

Which figure best represents the total amount of product in a catalysed reaction over a long period of time? (Let the horizontal axis represent time and the vertical axis represent amount of product.)

Question 4 (*Objective 2 (ii)*)

An enzyme may be rendered inoperative by:

(i) removing its product as fast as it appears;
(ii) blocking its active site;
(iii) halving its concentration;
(iv) doubling its concentration;
(v) adding coenzyme.

Categorize the pair of entities in Questions 5 and 6 according to the following key:

A (i) is *greater than* (ii)
B (i) is *less than* (ii)
C (i) is exactly or approximately *equal* to (ii)
D (i) may stand in *more than one of the above relations* to (ii)

Question 5 (*Objective 2* (*ii*))

(i) The effect or enzyme *concentration* on the *velocity* or a reaction.
(ii) The effect of enzyme concentration on the *equilibrium* of a reaction.

Question 6 (*Objective 2* (*ii*))

(i) The effect of *temperature* on the activation energy required for a given reaction.
(ii) The effect of a *catalyst* on the activation energy required for a given reaction.

Section 15.4

Question 7 (*Objective 3* (*i*))

In a reaction sequence in liver cells catalysed by a series of enzymes, the initial substrate was the 4-carbon acid we can represent arbitrarily B-O-A-T. The product found at the end of the reaction sequence was the 4 carbon acid, F-I-N-D. An investigator studying the sequence made some B-O-A-T in which the 'T' atom was radioactively labelled. After incubation with the liver cells, the radioactive substances F-O-O-T and F-O-N-T could be isolated. In the presence of an inhibitor, which prevented F-I-N-D being produced, the substances F-O-N-D and F-O-N-T accumulated. With another inhibitor, no F-O-N-D, F-O-N-T or F-I-N-D could be found, but C-O-O-T and C-O-A-T accumulated. On the understanding that each enzyme-catalysed reaction step involves the substitution of one letter in the word, and each intermediate must be a recognizable English word, devise a plausible reaction sequence leading from B-O-A-T to F-I-N-D.

Question 8 (*Objective 1* (*ii*))

Which best describes why adenosine triphosphate (ATP) is considered to be an 'energy currency' in organisms?

(i) ATP is one of the organic base compounds found in the DNA of all organisms.
(ii) Once formed, ATP is very stable and cannot be broken apart unless large amounts of energy are applied.
(iii) Phosphate is used in all organic chemical reactions.
(iv) ATP can be hydrolysed to ADP and inorganic phosphate.
(v) The third phosphate radical is easily transferred to other molecules making them reactive.

Question 9 (*Objective 2 (ii)*)

Which releases the greatest amount of usable energy per molecule of glucose broken down?

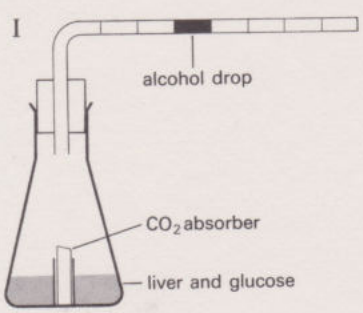
(i) Aerobic respiration in a muscle cell
(ii) Fermentation by a yeast cell
(iii) Glycolysis in a liver cell
(iv) Lactic acid formation in a muscle cell
(v) Synthesis of glycogen in a liver cell

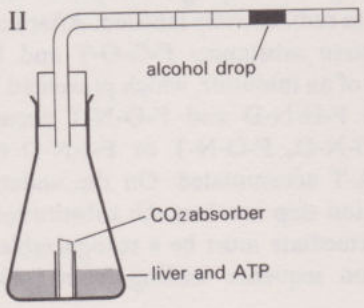
Question 10 (*Objective 1 (ii)*)

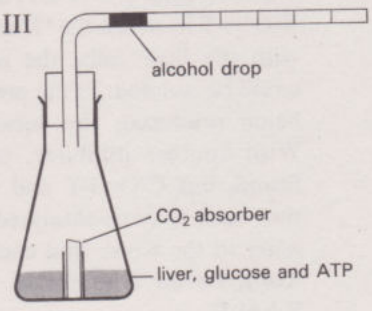
In respiration, pyruvic acid is:

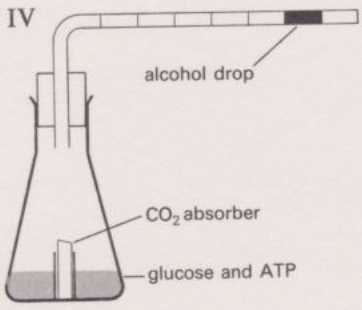
(i) one of the products of the Krebs cycle;
(ii) formed only if oxygen is not available;
(iii) used in the Krebs cycle;
(iv) involved in the transport of oxygen to the cell;
(v) responsible for the removal of carbon dioxide from the cell.

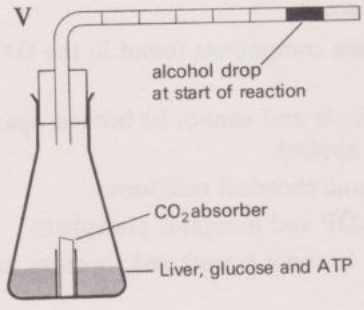
In an experiment, equal amounts of homogenized liver cells, with the ATP removed, are placed in Flasks I, II and III. Flask I contains glucose. Flask II contains a glucose solution and ATP. Flask IV contains no liver; only glucose and ATP. All flasks are gassed with oxygen and placed in water bath at 20° C. The following figures represent the flasks after one hour. (Drawing V shows the initial position of the drop in all four flasks.)











Question 11 (*Objective 2 (i) and 3 (i)*)

Which is the best interpretation from these data?

(i) ATP and glucose interact to produce CO_2.
(ii) ATP is necessary to start the respiratory breakdown of glucose.
(iii) Respiration cannot take place outside of living cells.
(iv) The Krebs cycle is not operating since no ATP is given off.
(v) No interpretation is possible.

Question 12 (*Objective 2 (i) and 3 (i)*)

What is the main function of flask IV?

(i) To measure gas changes due to temperature changes.
(ii) To measure gas changes due to temperature and air pressure changes.
(iii) To control changes in gas pressures.
(iv) To determine how glucose and ATP function.
(v) To account for cell enzymes.

Question 13 (*Objective 2 (i) and 3 (i)*)

The reaction in flask I stops after two hours. Which is the most likely explanation?

(i) All the ATP is used up.
(ii) All the glucose is used up.
(iii) All the CO_2 is used up.
(iv) Enzymes cannot function outside of living cells.
(v) Alcohol produced in fermentation denatures the enzyme.

Section 15.7.3

Question 14 (*Objective 2 (ii)*)

A hypothesis states that mitochondria contain the enzymes of the hydrogen transport chain bound to insoluble lipoprotein. Which observation most cogently supports this hypothesis?

(i) Mitochondria have a highly folded inner wall.
(ii) The space between the mitochondrial cristae is liquid-filled.
(iii) Ultrasonic disruption of mitochondria yields membrane fragments which can synthesize ATP.
(iv) Electron microscope examination of the membrane from mitochondria reveals the inner surface studded with granules.
(v) There are more mitochondria in muscle cells than in skin cells.

Question 15 (*Objective 2* (*ii*))

Certain yeast mutants lack 'normal' mitochondria.

They probably also lack:

(i) The ability to generate alcohol from glucose;
(ii) the capacity to synthesize fats;
(iii) the ability to form ATP by oxidative phosphorylation;
(iv) the enzyme which phosphorylates glucose;
(v) the capacity to be inhibited by iodoacetic acid.

Section 15.8

Question 16 (*Objective 1* (*ii*))

The oxygen liberated in photosynthesis results from the decomposition of:

(i) carbon dioxide;
(ii) carbohydrate;
(iii) ATP;
(iv) NADP;
(v) Water.

Question 17 (*Objective 2* (*ii*))

Respiration and photosynthesis both require which one of the following:

(i) Organic fuel.
(ii) Green cells.
(iii) Cytochromes.
(iv) Energy from carbon bonds.
(v) Sunlight.

Question 18 (*Objective 1* (*ii*))

Photosynthesis consists of essentially three biological reaction systems following in sequence. The third of these systems does which of the following?

(i) Traps light energy.
(ii) Synthesizes starch.
(iii) Fixes carbon dioxide.
(iv) Occurs in the nucleus.
(v) Works only in the presence of light.

Question 19 (*Objective 1* (*ii*))

The step in photosynthesis that directly requires light is the:

(i) excitation of chlorophyll;
(ii) transfer of hydrogen to NAD;

(iii) synthesis of ATP;
(iv) transfer of energy from chlorophyll to water;
(v) fixation of carbon dioxide.

Question 20 (*Objective 1* (*ii*))

It was once believed that the main function of photosynthesis was to purify the air. Our present-day view regarding the significance of photosynthesis is that it:

(i) converts light energy into chemical energy;
(ii) creates usable energy;
(iii) fixes CO_2 into carbohydrates;
(iv) reverses the action of respiration;
(v) splits water, releasing O_2.

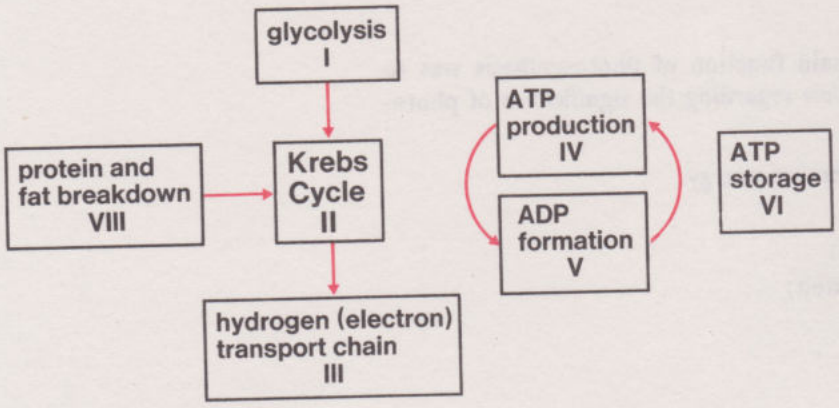
Question 21 (*Objective 1* (*ii*))

Which is the immediate energy source for the 'dark reaction' of photosynthesis?

(i) NAD.
(ii) ATP and $NADPH_2$.
(iii) ADP and NADP.
(iv) Light.
(v) CO_2 and H_2O.

Sections 15.6, 15.7 and 16.1

Glucose breakdown, respiration and ATP formation



The above flow chart represents, diagrammatically, the process of glucose breakdown and respiration in muscle cells.

Question 22 (*Objective 2* (*ii*))

Specify the links which should be added between ATP production box (iv) and the rest of the diagram:

1 (vi) ⟶ (iv)
2 (i) ⟶ (iv)
3 (ii) ⟶ (iv)
4 (iii) ⟶ (iv)

Question 23 (*Objective 2* (*ii*))

The greatest amount of oxidative phosphorylation occurs where?

1 in (i)
2 in (iii)
3 in (vii)
4 in (iv)
5 in (ii)

Question 24 (*Objective 2* (*ii*))

(ii) occurs in which organelle?

1 cell membrane
2 nucleus
3 mitochondria
4 ribosome
5 lysosome

Question 25 (*Objective 2 (ii)*)

(iii) occurs in which organelle?

1 cell membrane
2 nucleus
3 mitochondria
4 ribosome
5 lysosome

Question 26 (*Objective 2 (ii)*)

NAD plays a major role in which process?

1 (i)
2 (ii)
3 (iii)
4 (iv)
5 more than one of these

Question 27 (*Objective 2 (ii)*)

Which *cannot* occur in the absence ot oxygen?

1 (vii)
2 (ii)
3 (iii)
4 (iv)
5 more than one of these

Question 28 (*Objective 2 (ii)*)

When oxygen is not present, lactic acid accumulates in which process?

1 (ii)
2 (i)
3 (iii)
4 (v)
5 (iv)

Question 29 (*Objective 2 (ii)*)

Where do the cytochromes function?

1 (i)
2 (ii)
3 (iii)
4 (vii)
5 more than one of these

Section 16.3

The following experiment was designed to determine whether the 0.03 per cent of CO_2 present normally in the Earth's atmosphere is the most satisfactory (optimum) amount for the synthesis of carbohydrate in a plant.

A weighed geranium plant was placed in each of 10 containers. The containers were sealed and the CO_2 removed. CO_2 was then introduced beginning with 0.03 per cent and at intervals of 1 per cent (i.e. 1.03, 2.03 up to 9.03 per cent) twice each 24 hours. The plants were well watered at the outset and each container was kept on a greenhouse bench for three days. At the end of this period each plant was weighed. The questions that follow are designed to test your appreciation of experimental design in cell biology. Categorize each statement concerning this experiment according to the following key:

A This change is unimportant in the design of this experiment.

B This change would improve the experiment because it would provide a control, of one of the presently uncontrolled variables.

C This change would improve the experiment because it could aid in verifying the results.

D This change would improve the experiment because it would permit expression of results in quantitative terms.

Question 30 (*Objective 3 (i)*)

If only ten containers were available it would have been better to have kept five in continuous dark and five in continuous light.

Question 31

The interval of CO_2 should have been increased to allow certain containers to contain less than 0.03 per cent and others more than 9.03 per cent.

Question 32

The CO_2 content in each container should have been kept constant.

Question 33

The containers should have been kept in the same relative humidity.

Question 34

Several different benches should have been used.

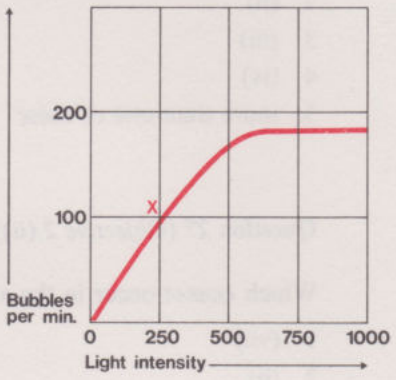
Question 35

The containers should all be made of Pyrex glass.

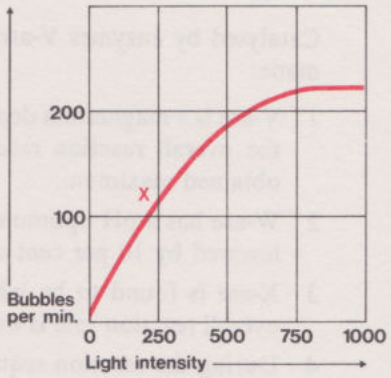
Question 36

The heights of the plants should have been measured at the beginning and at the end of the experiment.

Elodea (a green plant) shoots were immersed in water, illuminated, and allowed to photosynthesize. Bubbles of oxygen of constant size, which were emitted from the leaves and stem, were counted. Illumination was varied by moving the light source. Results are shown in the adjacent figure.



Question 37 (*Objectives 2 (i) and 2 (ii)*)



What is the best interpretation of these data?

1 Light is the limiting factor.
2 Light is limiting up to X when some other factor becomes limiting.
3 Some factor apart from light is limiting.
4 Some other factor is limiting up to X when light becomes limiting.

The above experiment was repeated except that a strong bicarbonate solution was added to provide an excess of CO_2. Results are shown in the adjacent figure.

Question 38 (*Objective 2 (i) and 2 (ii)*)

Which is the best interpretation of these data when compared to the previous experiment?

1 CO_2 is the limiting factor at X in experiment 1.
2 Light can be made to be limiting by decreasing other factors.
3 CO_2 is limiting at X in experiment 2.
4 Light was limiting at X in experiment 2.

Question 39

We often speak of 'rate limiting factors' in biology. Which of the following 'factors' would *not* limit photosynthesis, regardless of presence, absence or quantity?

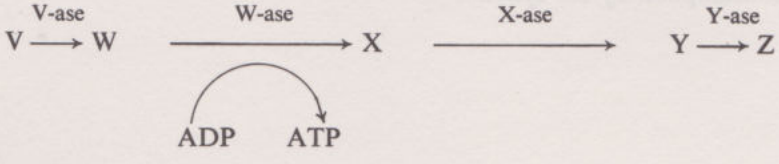
1 Oxygen.
2 Carbon dioxide.
3 Chlorophyll.
4 Water.
5 Light.

Question 40 (*Objective 1 (ii)*)

Which of the following structures is thought to be exceptionally rich in hydrolytic enzymes (nucleases, phosphates and proteases)?

1 Lysosomes.
2 Ribosomes.
3 Mitochondria.
4 Nuclei.
5 Cell membrane.

In a reaction sequence:



Catalysed by enzymes V-ase, W-ase, etc., the following observations are made:

1 V-ase is a magnesium dependent enzyme. In the absence of magnesium, the overall reaction rate is lowered by 5 per cent compared to the obtained maximum.

2 W-ase has a pH optimum of 7.4. At pH 7.0 the overall reaction rate is lowered by 10 per cent compared to the obtained maximum.

3 X-ase is found to be inhibited by Z. In the presence of 5mM Z the overall reaction rate is only 10 per cent of the maximum obtainable.

4 During the reaction sequence, ATP is synthesized from ADP as W is converted to X. Y-ase is found to be ATP dependent. Addition of an enzyme which hydrolyses ATP (ATP-ase) lowers the V → Z reaction rate to 80 per cent of the maximum obtainable.

Question 41 (*Objective 2* (*ii*))

Which is the rate-limiting reaction of the sequence?

1 V-ase.
2 W-ase.
3 X-ase.
4 Y-ase.

Question 42

The inhibition of X-ase by Z is called:

1 competitive inhibition;
2 an allosteric effect;
3 feedback inhibition;
4 repression.

Question 43

In an homogenized preparation of muscle tissue respiring with glucose as a substrate, which of the following factors are likely to be the major factor limiting the rate at which glucose is utilized?

1 uptake of glucose across the cell membrane;
2 presence of oxygen;
3 availability of inorganic phosphate;
4 presence of ATP.

Question 44

In the above preparation, respiring in an atmosphere of oxygen, an enzyme system splitting ATP (ATP-ase) is added. What effect is this likely to have on the rate of oxygen consumption by the system?

1 Increases.
2 Decreases.
3 Stays the same.
4 Rises at first then falls.

Question 45 (*Objectives 2 (i) and 3 (i)*)

In the same preparation, the mitochondria are removed by centrifugation, and the remaining material is allowed to respire with glucose as before. What effect is this likely to have on oxygen consumption?

1 Increases.
2 Decreases.
3 Stays the same.
4 Falls at first then rises.
5 One cannot predict.

Self-Assessment Answers and Comments

Question 1

(ii) is correct—see *The Chemistry of Life*, pp. 93–98. The exact chemical composition of the enzyme is irrelevant to the validity of the theory—nucleic acids could also form a 'lock and key'—at least in principle. The fact that enzymes are found in living organisms is irrelevant to the way they work.

Question 2

(ii) is the best representation—see *The Chemistry of Life*, pp. 91–92. If the substrate concentration is increased, a point is eventually arrived at, at which at any given time all the enzyme molecules are in contact with substrate molecules. Increasing the substrate concentration beyond this point will not result in a more rapid rate of reaction.

Question 3

(ii) See *The Chemistry of Life*, pp. 91–92. Eventually all the substrate will be catalysed: no more product can then be formed.

Question 4

(ii) Refer to section 15.3 and *The Chemistry of Life*, pp. 93–98. All the other procedures will alter the enzyme reaction rate, but they will not render it completely inactive.

Question 5

A is correct. An enzyme cannot affect the equilibrium of a reaction. See *The Chemistry of Life*, pp. 88–92.

Question 6

B is correct. The activation energy required for a reaction is not affected by temperature. Refer back, if you are not clear about this, to Unit 11.

Question 7

BOAT → COAT → COOT → FOOT → FONT → FOND → FIND. See *The Chemistry of Life*, pp. 102–103. But note, this *may* not be the unique solution. If you have produced another, you will be in the position of many experimental biochemists studying metabolic pathways.

Question 8

(v) is correct. See section 15.4. (i) (ii) and (iii) are factually incorrect. (iv) is correct but is not the relevant reaction in this context.

Question 9

(i) is correct. The type of cell in which the reactions occur is not relevant, we are only concerned with the reactions themselves; fermentation, glycolysis and lactic acid production are incomplete oxidations of glucose and yield only small quantities of energy. See *The Chemistry of Life*, pp. 127–135, and sections 15.6.3 and 15.6.4. Glycogen synthesis is an energy—not requiring an energy yielding reaction.

Question 10

(iii) is correct. See sections 15.6.4 and 15.6.5. (i), (iv) and (v) are factually incorrect. (ii) is wrong as pyruvic acid production from glucose occurs whether oxygen is present or absent. Only the subsequent *fate* of the pyruvic acid varies.

Question 11

(ii) is correct. Refer to section 15.6.1. The remaining answers are factually incorrect.

Question 12

(ii) is correct. Glucose and ATP do not in themselves cause changes in gas pressure, but changes in the temperature and pressure surrounding the flask would produce changes in position of the alcohol drop irrespective of enzyme-catalysed oxidation of glucose.

Question 13

(ii) is correct. Refer to section 15.6.1. (iv) and (v) are factually incorrect. (i) and (iii) cannot be right as the reactions of glucose oxidation *produce* ATP and CO_2.

Question 14

(iii) is the best answer. The other answers are correct, but not really relevant. Refer back to section 15.7.3.

Question 15

(iii) is correct. See sections 15.6.3 and 15.7.3. Alcohol is produced by fermentation in anaerobic conditions and is a cytoplasmic reaction: fat synthesis does not occur within the mitochondria. The initial phosphorylation of glucose during glycolysis is cytoplasmic and the site of iodoacetic acid inhibition (see 15.6.1. and 15.6.3) is during glycolysis, at the phosphoglyceraldehyde → phosphoglyceric acid site.

Question 16

(ii) is correct. See *The Chemistry of Life*, p. 242.

Question 17

(iii) is correct. See *The Chemistry of Life*, p. 243 and pp. 116–118. (i) is wrong because photosynthesis utilizes inorganic substances only as substrates. (ii) is wrong because respiration is a universal cellular activity not confined to green cells. (iv) is wrong because photosynthesis involves light energy not chemical bond energy. (v) is wrong because respiration can take place in the absence of light.

Question 18

(iii) is correct. See *The Chemistry of Life*, p. 242. The three critical steps are the splitting of water molecules, transfer of hydrogen to an acceptor and then reaction between the reduced acceptor molecule and CO_2. Light energy must obviously be trapped before it can take part in the water-splitting reactions, while starch synthesis may or may not occur in photosynthesizing cells. Light is not required for the fixation of CO_2.

Question 19

(i) is correct. See *The Chemistry of Life*, p. 243. All the other steps can occur in the dark.

Question 20

(i) is correct. See *The Chemistry of Life*, pp. 241 and 242. (ii) is wrong as energy cannot be created, whilst (iii) and (iv) are at best only partial answers to the question. (v) is correct.

Question 21

(ii) is correct. See *The Chemistry of Life*, p. 243. ADP and NADP do not generally function as sources of energy, in biological systems (it is ATP and $NADPH_2$ which do). (i) and (iii) are therefore wrong. (iv) is inappropriate as it is the dark reaction which is being considered though light is the ultimate energy source. (v) is incorrect as neither CO_2 nor H_2O can act as an energy source as such.

Question 22

2 and 4. If you were wrong refer to sections 15.6, 15.7 and 16.1.

Question 23

2.

Question 24

3.

Question 25

3.

Question 26

5.

Question 27

5.

Question 28

2.

Question 29

3.

If you were wrong, refer to sections 15.6, 15.7 and 16.1.

Question 30

A. Refer to sections 15.8 and 16.3. If you are uncertain about any of the suggested answers, refer back to *HED*.

Question 31

C.

Question 32

A.

Question 33

B.

Question 34

A.

Question 35

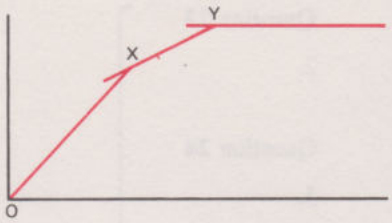
A.

Question 36

A.

Refer to sections 15.8 and 16.3. If you are uncertain about any of the suggested answers, refer back to *HED*.

Question 37



2 is the best interpretation of the data. To see why look at the curve again; it really consists of three parts:

(i) from the start to point X;
(ii) from X to Y;
(iii) from Y onwards.

During the first part the data shows that the rate of bubble production depends on the intensity of light being used. In this part of the experiment light availability limits bubbling rate. But in the curve from X onwards bubbling rate does not increase proportionally as the light intensity increases—so some other factor is limiting the reaction. Answer 1 is wrong therefore, because it doesn't fit the data completely. Similarly Answer 3 is wrong because light is obviously limiting at low intensities although not at higher light intensities (i.e. above part X in the curve).

Answer 4 is wrong because it involves a needless second limiting factor to account for the data up to point X and misinterprets the data from Y onwards where bubbling rate is independent of light intensity, not limited by it.

Question 38

1 is the best interpretation of those offered.

Answer 2 may be true but doesn't interpret the data provided. Answer 3 may be true but exceeds the available evidence. A further investigation using a different CO_2 concentration would be needed before this interpretation became acceptable. Answer 4 is incorrect as increasing the light intensity in experiment 2 does not proportionately increase bubbling rate. Some other factor is limiting the reaction.

Question 39

1 is correct because CO_2, H_2O and light are all used up in photosynthesis so if the amount of any one were limited then the rate of photosynthesis might be limited. The amount of chlorophyll available could limit the amount of light energy trapped by the cell and hence limit photosynthesis. But O_2 is not a substrate photosynthesis and so even if the amount of O_2 were limited the photosynthesis rate would not be affected.

Question 40

1 is correct. See *The Chemistry of Life*, p. 198.

Question 41

The rate-limiting step is the conversion of X → Y involving X-ase—so answer 3 is correct.

Compare the effects of absence of magnesium—maximum rate is 95 per cent of maximum possible.

Altering pH to 7.0 maximum rate is 80 per cent of maximum possible.

Inhibiting X-ase maximum rate is 10 per cent of maximum possible.

Adding ATP-ase—maximum rate is 80 per cent of maximum possible.

So the whole reaction sequence is much more sensitive to inhibition of X-ase and will be rate-limited at this point. See *The Chemistry of Life*, pp. 187–191.

Question 42

3 is correct. See *The Chemistry of Life*, pp. 196–198. Z is the end product of the sequence and inhibits X-ase. 1 is incorrect; for competitive inhibition to occur both substrate and inhibitor must compete for the active site of the enzyme. An allosteric effect may be involved in the inhibition by Z but there is no evidence offered on which to conclude this. (See *The Chemistry of Life*, p. 97.)

4 is not correct as repression implies the absence of a particular enzyme unless its substrate is present. See *The Chemistry of Life*, p. 197.

Question 43

2 is correct. The preparation is homogenized, so no cell membrane effect can be involved. Ⓟ and ATP levels could effect glucose metabolism but neither is of as great significance as oxygen. See *The Chemistry of Life*, p. 192.

Question 44

(A) is correct see *The Chemistry of Life*, pp. 192–196.

Removing the ATP as fast as it is formed by splitting it to ADP and Ⓟ will speed up the reactions which form ATP. If all the other parts of the system are not limited by a shortage of raw materials then the rate of ATP formation will rise. So 2 is incorrect. Only if the ATP formation were decoupled from the rest of the system outlined in Figure 23 (*The Chemistry of Life*, p. 192) would the rate of glucose oxidation remain unchanged after ATP had been added. This is presumably not the case here—so 3 is wrong.

4 might occur if after an initial increase in ATP production ATP-ase were inhibited or removed, or if some other part of the system outlined in Figure 23 became rate-limiting. But as long as ATP were present then ATP would be split whenever it was made and so there would be no reason to expect a decrease in rate after an initial increase. So 4 is wrong.

Question 45

2 is correct. From 15.7.3 the oxidative portion of glucose metabolism occurs in the mitochondria. Without them, the oxygen consumption will fall to practically zero.

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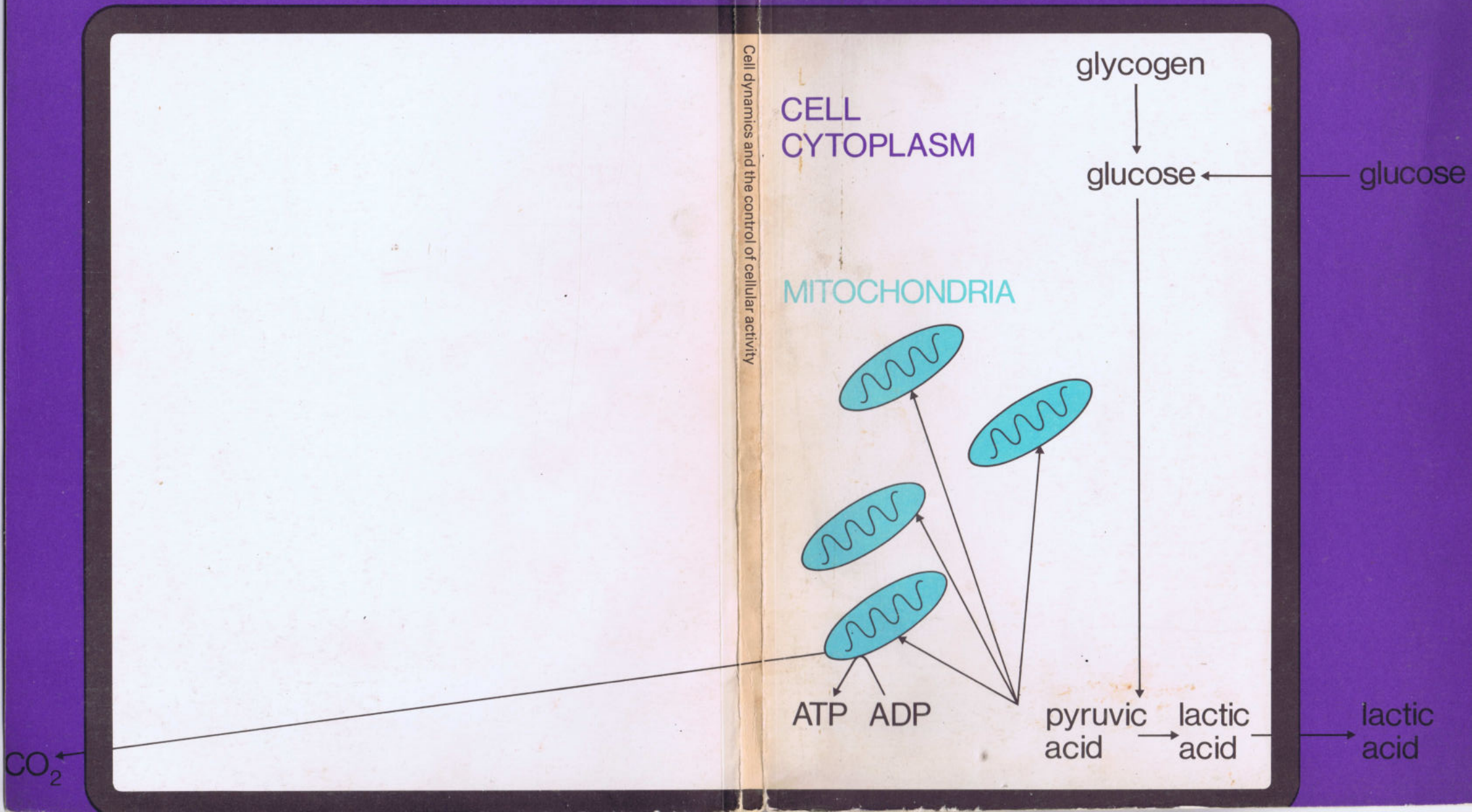

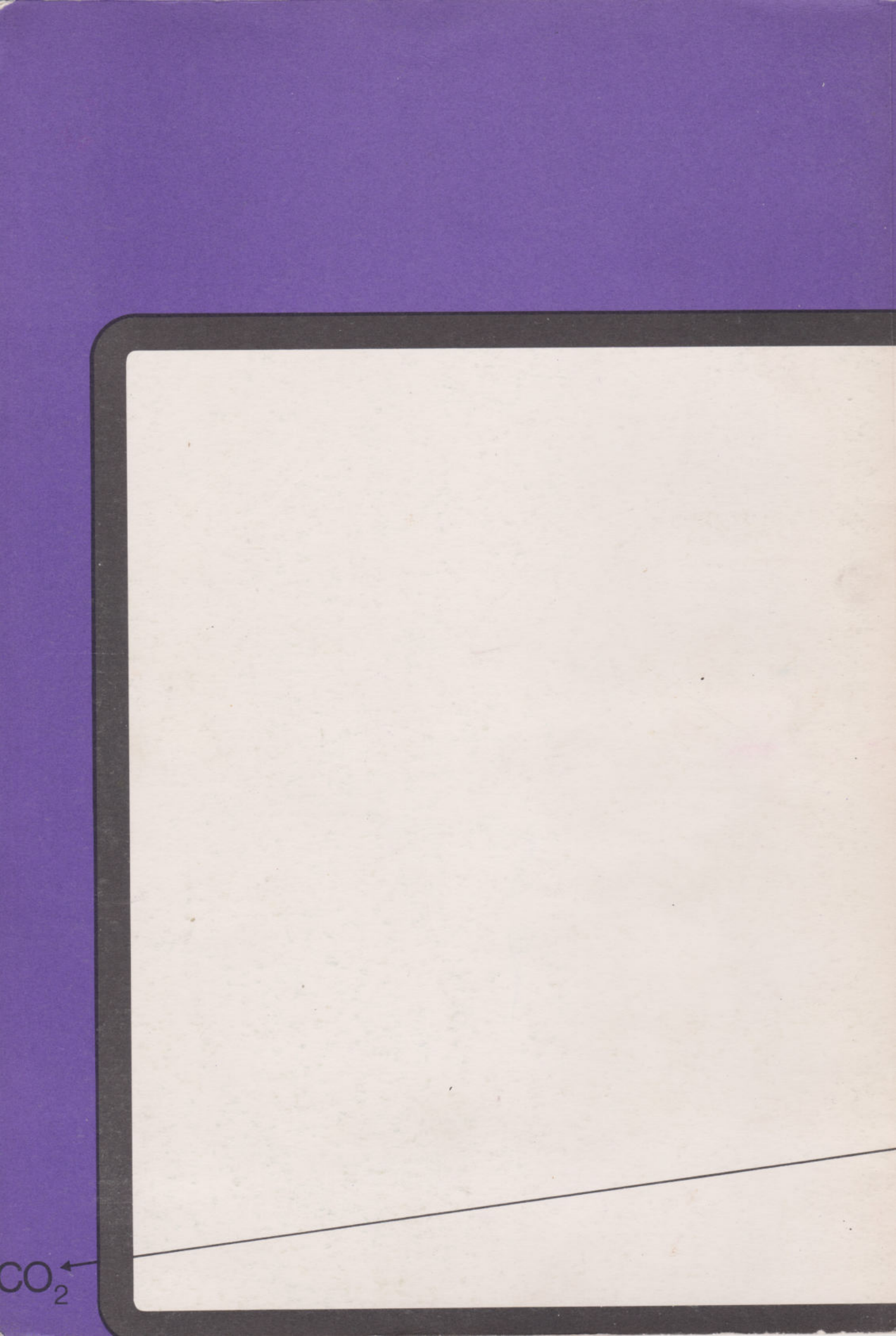
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